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The Effects of Thyroid Hormone on the HMG-CoA Reductase Gene Expression

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Abstract: The effects of the thyroid hormone (T_3) on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity were evaluated in a baby hamster kidney cell line, C100. The cells cultured in MEM were supplemented with 10% thyroid hormone-depleted fetal bovine serum (THDS-MEM) and had a 82.5% lower level of HMG-CoA reductase activity than the cells grown in a medium supplemented with fetal bovine serum (FBS-MEM). When T_3 was supplemented to THDS-MEM, the reduction of the reductase activity was blocked in a dose-dependent manner. In the cells grown in THDS-MEM containing T_3 at a concentration of 10^{-6} M, the level of HMG-CoA reductase activity was 91.8% relative to the cells grown in FBS-MEM. These changes in HMG-CoA reductase activity seemed to be at least partly due to the changes of HMG-CoA reductase mRNA levels. The level of HMG-CoA reductase mRNA in cells incubated in THDS-MEM decreased to 76.2% relative to the cells grown in FBS-MEM, while the level of reductase mRNA in cells incubated in THDS-MEM containing T_3 at a concentration of 10^{-6} M increased to 243.4% relative to the cells grown in FBS-MEM. The increase of HMG-CoA reductase mRNA level after T_3 treatment may have been due to the increased stability of reductase mRNA, because the transcriptional rate of the reductase gene did not change significantly in the presence or absence of T_3. These results indicate that T_3 stabilizes HMG-CoA reductase mRNA at the posttranscriptional level and regulates HMG-CoA reductase activity in a dose-dependent manner.

Key Words: gene expression, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thyroid hormone.

In vivo cholesterol biosynthesis is regulated by various factors such as nutritional factors, hormones, cholesterol and its metabolites. Among these factors, thyroid hormone has been known to exert important effects on cholesterol homeostasis. It has been reported that hypothyroidism is associated with high serum cholesterol/low density lipoprotein (LDL) levels and hyperthyroidism as being associated with decreased serum cholesterol levels (Dory and Roheim, 1981; Valdemarsson, 1983; Day *et al.*, 1989). T_4 treatment markedly decreased the level of total and LDL-cholesterol in hypothyroid patients, and methimasole treatment increased the level of serum cholesterol in hyperthroid patients (Engler and Riesen, 1993;Spandiro *et al.*, 1993).

The effects of the thyroid hormone on cholesterol homeostasis has been investigated extensively. At the transcriptional level, the thyroid hormone can regulate the expression of cholesterol 7-α hydroxylase (Crestani *et al.*, 1994), LDL receptor (Wiseman *et al.*, 1993), apo-B100 (Ness, 1991), and apo-A1 (Ness, 1991) genes. At the posttranslational level, the thyroid hormone can regulate the activity of cholesteryl ester transferase protein (Dullaart *et al.*, 1990), lecithin:cholesterol acyl transferase (Ruggiero *et al.*, 1990). In addition, the thyroid hormone can also increase bile acid synthesis and biliary secretion of cholesterol, which is one of the main metabolic pathways of cholesterol (Gebhard and Prigge, 1992). Although such regulatory roles of the thyroid hormone on the expression of various proteins involved in cholesterol homeostasis has been investigated extensively, the effects of thyroid hormone on the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase still remain unclear.

In animal tissue, HMG-CoA reductase is the rate-limiting enzyme for synthesis de novo of cholesterol. Specifically, HMG-CoA reductase synthesizes mevalonate from HMG-CoA; subsequently, mevalonate is either converted into cholesterol through multiple enzymic steps or used in the production of isoprenoids such as ubiquinone, dolichol, isopentenyl-tRNA and prenylated proteins (Goldstein and Brown, 1990).

When intracellular cholesterol levels increase, the activity of HMG-CoA reductase is suppressed to prevent

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the over-accumulation of cholesterol within the cells (Goldstein and Brown, 1977; Brown and Goldstein, 1980; Rudney and Sexton, 1986). It has been well established that an excess amount of cholesterol or sterols can suppress endogenous cholesterol synthesis by suppressing HMG-CoA reductase activity at the transcriptional, posttranscriptional, translational, and post-translational levels.

In CHO cells, the cholesterol component of LDL decreased HMG-CoA reductase activity primarily by reducing its mRNA level (Chin *et al.*, 1984). This effect of LDL was mediated through an octameric nucleotide sequence in the 5′ promoter region known as the sterol regulatory element or SRE (Reynolds *et al.*, 1984; Osborne *et al.*, 1985; Osborne *et al.*, 1988). Mevalonate, which is the product of HMG-CoA reductase and the precursor of various isoprenoids and sterols, can also decrease the HMG-CoA reductase mRNA level (Clarke *et al.*, 1983). Additionally, it was reported that mevalonate or its metabolites inhibited the translation of HMG-CoA reductase mRNA to decrease *de novo* synthesis of cholesterol further (Tanaka *et al.*, 1983; Peffley and Sinensky, 1985; Nakanishi *et al.*, 1988; Peffley, 1992).

Choi *et al.* (1993) reported that inhibition of protein/RNA synthesis blocked 25-hydroxycholesterol-mediated suppression of HMG-CoA reductase mRNA in lovastatin-treated hamster cells at a posttranscriptional level. The suppressive effect of 25-hydroxycholesterol was mediated by a certain regulatory element in the 3′-untranslated region of HMG-CoA reductase mRNA (Choi and Peffley, 1995). In the presence of an excess amount of cholesterol, phosphorylation of HMG-CoA reductase has been proposed to induce the accelerated degradation and the inactivation of HMG-CoA reductase. Miller *et al.* (1989) proposed that phosphorylation of the linker domain of HMG-CoA reductase was important for the degradation of this enzyme. This linker domain contains the PEST sequence which was identified in the eucaryotic proteins that were rapidly degraded (Rogers *et al.*, 1986; Dice, 1987; Miller *et al.*, 1989). The membrane spanning region of HMG-CoA reductase also appears to play an important role in the regulation of the activity and degradation of HMG-CoA reductase (Gil *et al.*, 1985). In rat livers, about 80~90% of the total HMG-CoA reductase is in the inactive phosphorylated form (Brown *et al.*, 1979).

The effects of the thyroid hormone on cholesterol biosynthesis still remain unclear. Although it was reported that thyroid hormone treatment markedly increased the level of HMG-CoA reductase mRNA in the hypophysectomized rat liver, it is not clear whether thyroid hormone directly increases the transcriptional rate or mRNA stability or indirectly by decreasing intrahepatic cholesterol pool (Sample *et al.*, 1987; Simonet and Ness, 1988; Ness *et al.*, 1990). It is difficult to elucidate the exact mechanism of thyroid hormone *in vivo* because the expression of HMG-CoA reductase gene is regulated by various physiological and humoral factors. Various factors such as dietary factors (Rudney and Sexton, 1986; Connor and Connor, 1990) or increased synthesis of bile acid (Day *et al.*, 1989; Gebhard and Prigge, 1992) can regulate cholesterol biosynthesis. Additionally, it is very hard to deduce the specific effect of thyroid hormone from the data produced from the complex interplay of various hormones. The relative importance of various regulators especially remain uncertain (Lakshmana *et al.*, 1975). For example, it has been reported that estrogen, testosterone, or glucocorticoids, could suppress the reductase activity (Brown and Goldstein, 1974; Lakshamana *et al.*, 1975). The aim of this experiment was to elucidate the role and possible mechanisms of the thyroid hormone on cholesterol biosynthesis by using an *in vitro* cell culture system and baby-hamster kidney C100 cells.

Materials and Methods

Cell culture

C100 cells were provided by Dr. Robert Simoni, Stanford School of Medicine. The cells were maintained in Minimal Essential Medium (MEM) containing 5% (v/v) fetal bovine serum (FBS) at 37℃. For experimental purposes, the cells were cultured in MEM containing 5% (v/v) thyroid hormone-depleted fetal bovine serum (THDS). THDS was prepared from whole FBS as described by Samuels *et al.* (1988). To prepare THDS, FBS retaining AG-1X-anion exchange resin (Bio-Rad, Richmond, USA) at a concentration of 50 mg/ml was incubated at room temperature for 5 h. After removing the resin by centrifugation at 1,000×*g* for 10 min, the serum retaining additional resin at a concentration of 50 mg/ml was incubated at room temperature for further 20 h. After removing the resin by centrifugation, the serum was sterilized by filtration through a 0.2 μm filter. The removal rate of T_3 was 95% when estimated by using $[^{125}I]T_3$. T_3 was purchased from Sigma Co. (St. Louis, USA). The stock of T_3 was prepared in the normal saline, pH 10.0, at a final concentration of 10^{-2} M and stored at −70℃.

RNA isolation

The single step method of RNA isolation by acid guanidinium thiocyanate/phenol/chloroform extraction, as described by Chomczynski and Sacchi (1987), was used throughout this study. The cells were lysed in

a solution containing 4 M guanidinium thiocyanate, 2.5 mM sodium citrate (pH 7.0), 0.5% sodium lauryl sarcosyl, and 0.1 M 2-mercaptoethanol. The cell lysate was made 0.2 M with respect to sodium acetate, pH 4.0. After adding a 1/5 volume of chloroform, the total RNA was extracted with 1 volume of water-saturated acid phenol, pH 5.0. After precipitation with 2-propanol, pelleted RNA was washed with 80% ethanol, vacuum dried, and resuspended in 0.05 M Tris-HCl, pH 7.5, 0.01 M $MgCl_2$ and 0.05 mg/ml acetylated BSA containing 6 μg of RNase-free DNase and 10 U of RNasin (both from United States Biochemicals, USA). The samples were incubated at 37℃ for 45 min, and the RNA was extracted by using acid phenol as described. RNA was precipitated with ethanol, dried under a vacuum, and resuspended in diethyl pyrocarbonate-treated water. The samples were stored at −70℃.

RNA slot blot assay

The samples of total RNA ranging from 4 to 16 μg were denatured with 6 M formaldehyde in 10×SSC for 15 min at 65℃. The SSC was composed of 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0. The denatured samples were transferred to the nitrocellulose membrane. After baking the membrane at 80℃ for 2 h, the membrane was prehybridized for 16 h at 42℃ with a hybridization buffer. The hybridization buffer was composed of 50% formamide, 6×SSC, 5×Denhardt's solution, 0.5% SDS, 50 mM $NaPO_4$, and 100 μg/ml of sheared salmon sperm DNA. Cloned cDNA probes were labelled with [α-^{32}P]dCTP to a specific radioactivity of at least 10^9 cpm/μg by using random priming kit (Promega, USA). The membrane was subsequently hybridized with ^{32}P-labelled probes which was prepared from a 2.5 kb BglII cDNA insert of pRed227. The plasmid, pRed227, contained a sequence homologous to Chinese hamster HMG-CoA reductase cDNA (Chin *et al.*, 1984). Hybridization and washing conditions were done as described previously (Peffley, 1992). The membranes were dried and exposed to X-ray film (Kodak, USA) at −70℃ for 2 days. As a control, the membrane was hybridized with ^{32}P-labeled pHFβA-1, a plasmid containing a sequence homologous to human β-actin cDNA (Ponte *et al.*, 1984). Levels of HMG-CoA reductase and β-actin mRNA were estimated by scanning densitometry of the autoradiographs prepared from such membranes (LKB densitometer).

Transcriptional run-on assay

The relative transcriptional rate of HMG-CoA reductase, β-actin mRNAs was determined on isolated nuclei using the method described by Celano *et al.* (1989). For each experimental treatment, approximately 5×10^7 cells were lysed in 2 ml of a lysis buffer. The lysis buffer was composed of 20 mM Tris-Cl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, and 0.5% Nonidet P-40. The lysate was disrupted in a glass homogenizer with a loose fitting pestle and nuclei were pelleted by centrifugation at 500×*g* for 5 min at 4℃. The nuclei were washed once with a lysis buffer lacking Nonidet P-40 and pelleted. The nuclear pellets were resuspended in a glycerol storage buffer containing 50 mM Tris-Cl, pH 8.0, 40% glycerol, 5 mM $MgCl_2$, and 0.1 mM EDTA. The samples were frozen and stored at −70℃. For *in vitro* transcription reactions, the nuclei stored at −70℃ were thawed on ice and then incubated at 26℃ for 30 min in a 1 volume of 2× reaction buffer containing 10 mM Tris-Cl, pH 8.0, 0.3 M KCl, 5 mM $MgCl_2$, 5 mM DTT, 1 mM each of ATP, GTP, CTP and 100 μCi of [γ-^{32}P]UTP (specific activity, 800 μCi/mmol). The reaction was terminated by adding a solution containing 10 mM Tris-Cl, pH 7.4, 0.5 M NaCl, 50 mM $MgCl_2$, 2 mM $CaCl_2$, and 40 μg of RNase-free DNase followed by incubation for 5 min at 26℃. To each sample, 200 μl of a buffer containing 0.5 M Tris-Cl, pH 7.4, 0.125 M EDTA, pH 8.0, and 5% SDS, was added. The samples were incubated with 200 μg of Proteinase K at 42℃ for 30 min. The RNA was extracted with phenol: chloroform (1 : 1, v:v) and approximately 2×10^6 cpm of ^{32}P-labeled RNA was added to a 1 ml of hybridization buffer. The RNA was hybridized to slot-blots containing pRed227, pHFβA-1, and pBR322 plasmids. The hybridization was done at 42℃ for 48 h. The blots were washed sequentially in 2×SSC, 0.1% SDS and 0.1×SSC, 0.1% SDS, dried and exposed to X-ray film at −70℃ for 3 days.

HMG-CoA reductase assay

The cell-free extracts were prepared as described by Kaneko *et al.* (1978). The cells were washed once with a ice-cold buffer composing 50 mM Tris-HCl, pH 7.4 and 150 mM NaCl and centrifuged at 900×*g* for 5 min at 4℃. The cell pellets were lysed in a buffer retaining 50 mM potassium phosphate, pH 7.4, 5 mM DTT, 5 mM EDTA, 200 mM KCl, and 0.25% (v/v) Brij96 (Sigma, USA). Microsomal reductase activity was assayed essentially as described by Shefer *et al.* (1972). The 0.4 ml reaction mixture contained 100 mM sucrose, 50 mM KCl, 40 mM potassium phosphate, pH 7.2, 30 mM potassium EDTA, pH 7.2, 10 mM DTT, 7 mM NaCl, 2 mM $NADP^+$, 10 mM glucose 6-phosphate, 1 U glucose 6-phosphate dehydrogenase, 174 μM DL-[3-^{14}C] HMG-CoA (3,000~4,000 dpm/nmol), and 400~1,000 μg protein. The mixture lacking DL-[3-^{14}C]HMG-CoA was preincubated for 20 min at 37℃. After adding DL-[3-^{14}C]HMG-CoA, the reaction

mixture was incubated at 37℃ for 30 min. After adding 1 mg of mevalonolactone, 40 μl of 33% KOH, 30,000 dpm of [5-^{3}H]mevalonolactone, and 84 μl of 5 N HCl, the reaction mixture was incubated at 37℃ for further 30 min After centrifuging the sample at a microcentrifuge (Eppendorf, USA) for 5 min, the supernatant was prepared. Each 100 μl of supernatant was applied on a silica gel TLC plate (Eastman, USA) in a solvent system of acetone-benzene (1 : 1, v : v). The area corresponding to a Rf of 0.55~0.8 was scraped and counted for radioactivity by using scintillation spectrometer (Beckmann, USA).

Results

The effect of thyroid hormone-depletion on HMG-CoA reductase activity in C100 cells

The HMG-CoA reductase activity decreased steadily when C100 cells were cultured in THDS-MEM (Fig. 1). After 6 h of incubation, HMG-CoA reductase activity decreased to the level of 67.8% relative to the cells grown in FBS-MEM. After 24 h of incubation, HMG-CoA reductase activity decreased to the basal level, which had activity of 17.5% of those cells grown in FBS-MEM. As shown in Fig. 1, HMG-CoA reductase activity remained at the basal level until 72 h after treatment.

T_3 effects on HMG-CoA reductase activity

When the cells were grown in THDS-MEM containing T_3, the reduction of HMG-CoA reductase activity was inhibited in a dose-dependent manner (Fig. 2). Even at the lowest concentration of T_3 (10^{-9} M) used in this study, the level of HMG-CoA reductase activity was 183% relative to the cells grown in THDS-MEM. In the cells grown in THDS-MEM containing T_3 at a concentration of 10^{-6} M, the level of HMG-CoA reductase activity was 328% relative to the cells grown in THDS-MEM and 91.8% relative to the cells grown in FBS-MEM, which indicated that depletion of the thyroid hormone induced reduction of HMG-CoA reductase activity in C100 cells.

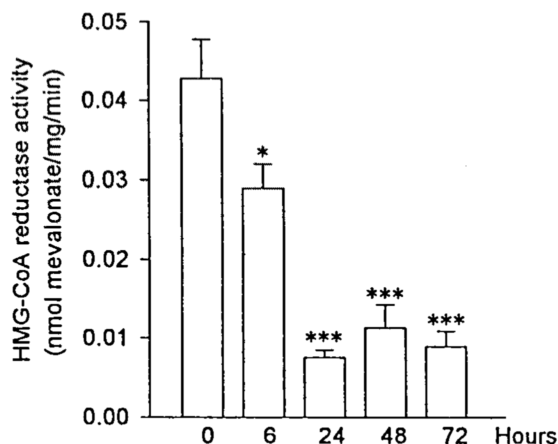

Fig. 1. The time course for reduction of HMG-CoA reductase activity in C100 cells incubated in THDS-MEM. Cells were grown in FBS-MEM after which the medium was changed to MEM supplemented with THDS. Cells were incubated for the indicated time intervals, and HMG-CoA reductase activity was measured as described in the Materials and Methods. The values represent means± S.E. of at least three independent experiments. *Asterisks* indicate significant differences as compared to 0-h controls incubated in FBS-MEM only (*p<0.05, ***p<0.001 by Student's t-test).

T_3 increases HMG-CoA reductase activity in a time-dependent manner

The addition of T_3 to cells pre-incubated in THDS-MEM for 48 h rapidly increased HMG-CoA reductase activity (Fig. 3). After adding T_3 with a final concentration of 10^{-6} M to the medium, HMG-CoA reductase activity increased within 2 h and reached a 77.1% level relative to the cells grown in FBS-MEM after 6 h of treatment, which indicated that T_3 could induce the reductase activity.

T_3 increases the level of HMG-CoA reductase mRNA

The level of HMG-CoA reductase mRNA in cells incubated in THDS-MEM containing T_3 at a concentra-

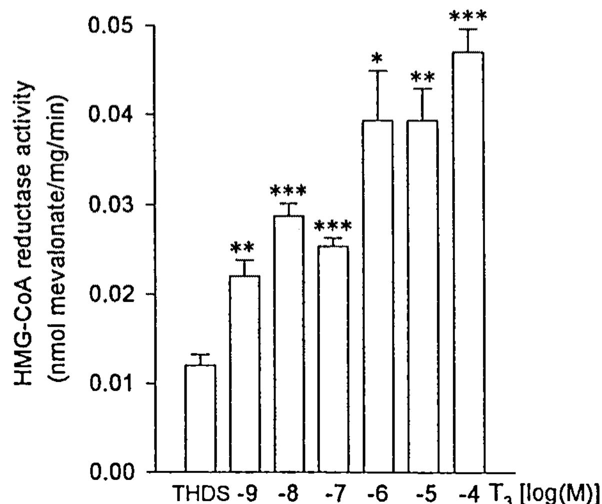

Fig. 2. T_3 inhibits the reduction of HMG-CoA reductase activity induced by thyroid hormone depletion in a dose-dependent manner. C100 cells were grown in FBS-MEM after which the medium was changed to THDS-MEM. T_3 was added to an appropriate concentration and cells were incubated for a further 48 h. After incubation, HMG-CoA reductase activity was measured as described in the Materials and Methods. The values represent means± S.E. of at least three independent experiments. Asterisks indicate significant differences as compared to THDS controls incubated in THDS-MEM only (*p<0.05, **p<001, ***p<0.001 by Student's t-test).

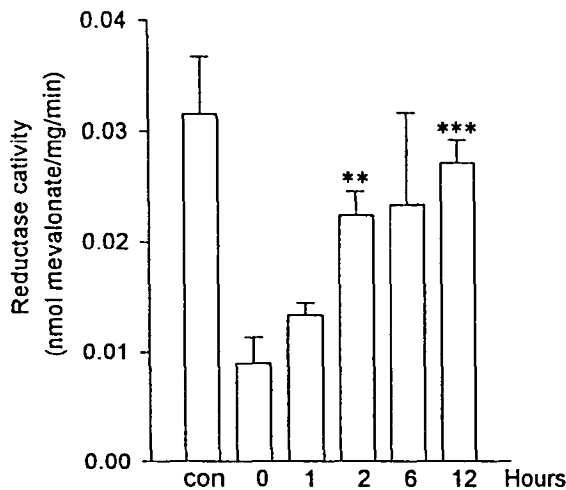

Fig. 3. The time course for the increase of HMG-CoA reductase activity in C100 cells after T_3 treatment. Cells were incubated for 48 h in THDS-MEM. The medium was then supplemented with T_3 to a final concentration of 10^{-6} M and cells were incubated for the indicated time periods. As the control (CON), cells were grown in FBS-MEM. After incubation, HMG-CoA reductase activity was measured as described in the Materials and Methods. The values represent means± S.E. of at least three independent experiments. Asterisks indicate significant differences as compared to 0-h controls incubated in THDS-MEM only (**$p<0.01$, ***$p<0.001$ by Student's t-test).

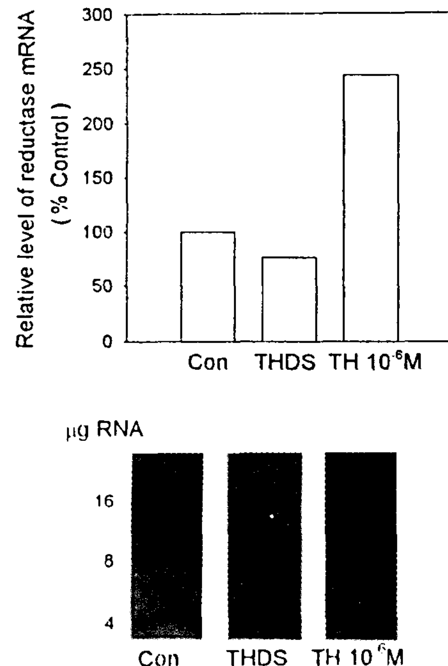

Fig. 4. The effects of thyroid hormone (T_3) on the levels of HMG-CoA reductase mRNA. C100 cells were incubated in FBS-MEM (CON), THDS-MEM (THDS), or THDS-MEM containing T_3 at a concentration of 10^{-6} M (TH 10^{-6} M). Cells were incubated for 48 h and total RNA was extracted. Levels of HMG-CoA reductase and β-actin mRNAs were estimated by slot blot assay. The relative levels of HMG-CoA reductase and β-actin mRNAs in C 100 cells grown in FBS-MEM were set at 100%.

tion of 10^{-6} M increased to 243.4% relative to the cells grown in FBS-MEM, while the level of HMG-CoA reductase mRNA in cells grown in THDS-MEM alone decreased slightly to 76.2% relative to the cells grown in FBS-MEM (Fig.4). These results indicated that T_3 could regulate HMG-CoA reductase activity at the mRNA level.

The effect of T_3 on the transcriptional rate of HMG-CoA reductase gene

The presence or absence of T_3 in the medium did not affect the transcriptional rate of the HMG-CoA reductase gene significantly (Fig. 5). In addition, the transcriptional rate of the HMG-CoA reductase gene did not change significantly when C100 cells cultured in THDS-MEM for 48 h were treated with T_3 at a concentration of 10^{-6} M (Fig. 6). These results indicated that T_3 increased the level of HMG-CoA reductase mRNA by stabilizing it.

Discussion

This study using baby hamster kidney cell line, C100 cells, demonstrated that endogenous cholesterol biosynthesis was suppressed in the absence of the thyroid hormone. In the presence of the thyroid hormone, HMG-CoA reductase activity was retrieved to the control level. Although similar results had been reported in *in vivo* experiments (Sample *et al.*, 1987; Simonet and Ness, 1988) using hypothyroid or hypophysectomized rats, it had not been made clear whether or not the thyroid hormone directly increased HMG-CoA reductase activity. Thyroid hormone treatment could decrease the high serum cholesterol/LDL levels associated with hypothyroidism, which might induce the increase in HMG-CoA reductase activity (Dory and Roheim, 1981; Valdemarsson, 1983).

In the liver, bile flow plays a pivotal rôle in the regulation of intracellular cholesterol homeostasis. It has been reported that T_3 increased bile flow and biliary cholesterol output, which preceded the rise of HMG-CoA reductase activity (Day *et al.*, 1989; Packard *et al.*, 1993). Increased bile acid synthesis and secretion of biliary cholesterol should decrease the intrahepatic cholesterol pool, which was suggested as the primary action of thyroid hormone on HMG-CoA reductase activity (Ness *et al.*, 1990; Packard *et al.*, 1993). However the results from this study suggest that the thyroid hormone directly increased the de novo synthesis of cholesterol, because C100 cell line was derived from the baby hamster kidney cells.

At 6 h after incubating C100 cells in THDS-MEM, HMG-CoA reductase activity decreased to the level of

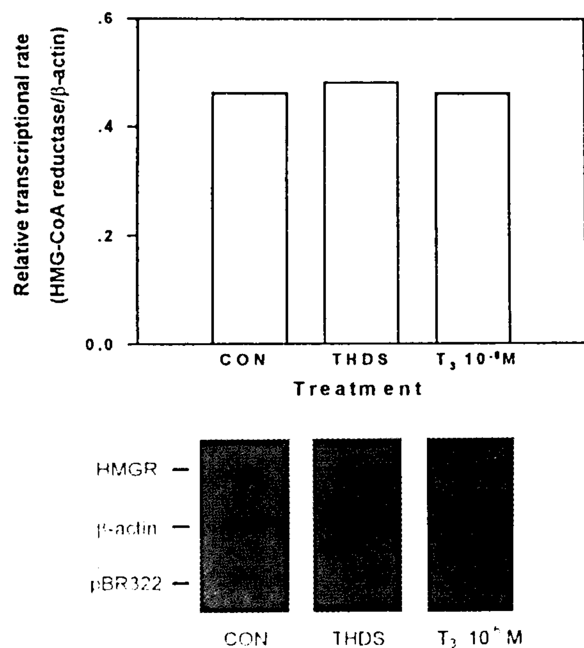

Fig. 5. The effects of T_3 depletion on the transcriptional rate of HMG-CoA reductase gene. C100 cells were incubated in FBS-MEM (CON), THDS-MEM (THDS), or THDS-MEM containing T_3 at a concentration of 10^{-6} M (TH 10^{-6} M). After 48 h, cells were harvested and a transcriptional nuclear run-off assay was done as described in the Materials and Methods. In each of these experiments, 5 μg of plasmid was used.

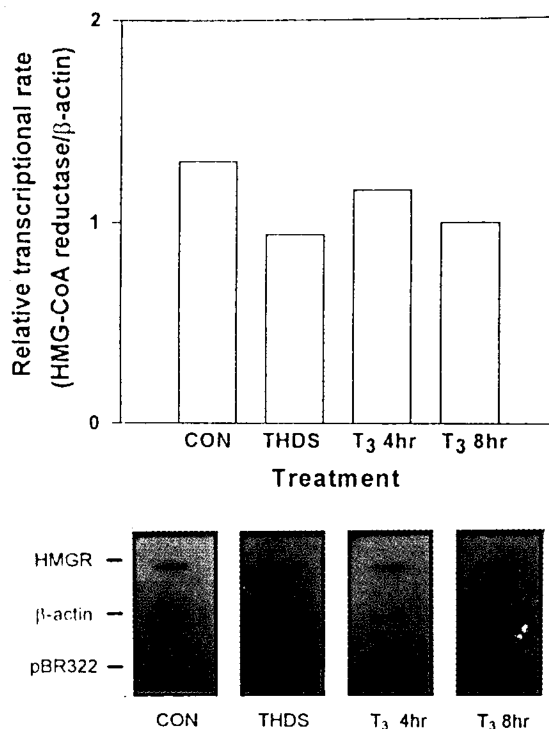

Fig. 6. The effects of T_3 replacement on the transcriptional rate of HMG-CoA reductase gene. C100 cells were incubated for 48 h in THDS-MEM. The medium was then supplemented with T_3 to a final concentration of 10^{-6} M and cells were incubated for a further 4 h (T_3 4 h) and 8 h (T_3 8hr). As the controls, cells were grown in FBS-MEM (CON) or cells were grown in THDS-MEM only (THDS). After incubation, cells were harvested and a transcriptional nuclear run-off assay was done as described in the Materials and Methods. In each of these experiments, 5 μg of plasmids was used except pHFβA-1. One μg of pHFβA-1 was used.

67.8% relative to the control level. This result suggests that the decrease of reductase activity depended on the degradation rate of HMG-CoA reductase, because the half-life of HMG-CoA reductase was estimated at approximately 8.4 h (Edward *et al.*, 1983; Liscum *et al.*, 1985; Chun *et al.*, 1990). Addition of T_3 to C100 cells pre-incubated in THDS-MEM for 48 h rapidly increased HMG-CoA reductase activity within 2 h. This result shows that the increase in HMG-CoA reductase activity precedes the increase in bile flow after T_3 treatment. It had been reported that the increase in bile flow initiated at 12 h and the increase in bile acid synthesis initiated at 24 h after T_3 treatment (Gebhard and Prigge, 1992).

In the cells treated with the thyroid hormone at a concentration of 10^{-6} M, the level of HMG-CoA reductase mRNA increased to double the number of cells cultured in FBS-MEM, as compared to thyroid hormone-depletion which slightly suppressed the level of reductase mRNA. The data from nuclear transcriptional run-on assays showed that the thyroid hormone did not change the transcriptional rate of HMG-CoA reductase gene significantly in C100 cells. These results indicate that the thyroid hormone could stabilize HMG-CoA reductase mRNA at the posttranscriptional level. Simonet and Ness (1988) reported that feeding hypophysectomized rats a diet containing porcine thyroid powder increased the level of hepatic HMG-CoA reductase mRNA 20~40 fold, and increased the half life of it. The half life of hepatic HMG-CoA reductase mRNA from hypophysectomized rats was about 3 h, equal to that of normal rats, and it was increased to 12 h if the hypophysectomized rats were pre-treated with thyroid hormone (Sample *et al.*, 1987; Simonet and Ness, 1988). They suggested that the effects of thyroid hormone may be mediated by a primary effect on cholesterol 7-α hydroxylase gene expression (Ness *et al.*, 1990). Cholesterol 7-α hydroxylase is the rate-limiting enzyme in bile acid synthesis. However, the results from this study suggest that the thyroid hormone may directly stabilize HMG-CoA reductase mRNA by changing the levels of certain regulatory factors. The negative feedback regulation of HMG-CoA reductase mRNA stability by sterols had been reported. Choi *et al.* (1993) reported that continuous protein/RNA synthesis was necessary for the 25-hydroxycholesterol-mediated suppression of HMG-CoA reductase mRNA levels. And a certain regulatory element in the

3′ untranslated region of HMG-CoA reductase mRNA mediated such suppressive effect of 25-hydroxycholesterol (Choi and Peffley, 1995). It is not still clear whether or not the thyroid hormone can countervail the suppressive effect of 25-hydroxycholesterol.

Although Simonet and Ness (1988) reported that thyroid hormone treatment increased the transcriptional rate of HMG-CoA reductase gene about 5 fold in hypophysectomized rat liver, such effect of the thyroid hormone was not observed in this study. The results from this study suggest that the thyroid hormone increased the transcriptional rate of HMG-CoA reductase gene by decreasing the intracellular cholesterol pool as described previously (Day *et al.*, 1989; Packard *et al.*, 1993; Ness *et al.*, 1990).

It is interesting to note that the thyroid hormone at a concentration of 10^{-6} M increased the level of HMG-CoA reductase mRNA to double the number of the cells cultured in FBS-MEM, while it did not increase HMG-CoA reductase activity higher than that of those cells cultured in FBS-MEM. In addition, the transcriptional rate of HMG-CoA reductase gene and the level of HMG-CoA reductase mRNA did not decrease markedly in the absence of thyroid hormone when the reductase activity decreased to 17.5% relative to the control level. The discrepancy between HMG-CoA reductase mRNA levels and its activity may be due to differing feedback controls. It had been reported that the translation of HMG-CoA reductase mRNA was regulated by mevalonate and its metabolites (Tanaka *et* al., 1983; Peffley and Sinensky, 1985; Nakanishi *et al.*, 1988; Peffley, 1992). It had been also reported that the activity and stability of HMG-CoA reductase can be regulated at the posttranslational levels by phosphorylation of this enzyme or change in the physical property of the membrane in which this enzyme is embedded (Gil *et al.*, 1985; Miller *et al.*, 1989). In rat liver, about 80~90% of the total HMG-CoA reductase was in the inactive phosphorylated form when estimated by using phosphatase inhibitor, NaF, or *E. coli* alkaline phosphatase (Brown *et al.*, 1979).

The state of hypothyroidism may have marked influence on intracellular cholesterol homeostasis due to both suppressed HMG-CoA reductase activity and suppressed transport of LDL-cholesterol. The genes of the LDL receptor and HMG-CoA reductase are regulated in a similar fashion (Van der Westhuyzen *et al.*, 1990). Such changes in cholesterol homeostasis may influence the physical properties of various cellular membranes which can further impair the activities of the membrane-bound enzymes. For examples, Pilarska *et al.* (1991) reported that the thyroid hormone changed the fluidity of the sarcolemma membrane of skeletal muscle and the activity of calmodulin-stimulated Ca^{2+}-Mg^{2+}-ATPase. Paradies *et al.* (1991) also reported that the thyroid hormone changed the lipid composition of rat liver mitochondria. The results from this study suggest that the thyroid hormone may play an important role in maintaining the physical properties of cellular membranes and the activities of membrane-bound enzymes.

In conclusion, the results from this study indicate that T_3 stabilizes HMG-CoA reductase mRNA at the post-transcriptional level and regulates HMG-CoA reductase activity in a dose-dependent manner.

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