Plasma Cholesteryl Ester Transfer Protein Activity Is High in Infants and Is Not Affected by Thyroid Hormones

Tadashi Asami, Masaki Wada, and Makoto Uchiyama

We investigated the effects of thyroid dysfunction on cholesteryl ester transfer protein (CETP) by studying plasma CETP activity in hypothyroid infants before and after they were rendered euthyroid by L-thyroxine (LT_4) replacement therapy. To exclude environmental factors possibly affecting plasma CETP activity, we selected hypothyroid infants to study plasma CETP activity. Plasma CETP activity was measured as the rate of radiolabeled cholesteryl ester transfer from high-density lipoprotein (HDL) to serum apolipoprotein B (apo B)–containing lipoproteins in plasma from 14 hypothyroid infants before and 2 months after LT_4 replacement, 23 normal infants, and 61 normal adults. Relationships between CETP and thyroid hormones were examined separately in the 14 hypothyroid infants and 32 euthyroid infants, including the 14 above-described hypothyroid infants and an additional 18 treated hypothyroid infants. Serum levels of thyrotropin (TSH), free thyroxine (FT_4), and free triiodothyronine (FT_3) were also determined on an autoanalyzer system in our hospital. In contrast to previous reports, we found no differences in plasma CETP activity between hypothyroid infants and age-matched normal infants. LT_4 substitution did not cause any changes in plasma CETP activity after therapy. Plasma CETP activity showed no correlation with serum TSH, FT_4, and FT_3 levels. Both hypothyroid and normal infants were found to have significantly higher plasma CETP activity than normal adults. From these results, we conclude that in infants thyroid hormones do not affect plasma CETP activity, and normal infants have high plasma CETP activity, compared with normal adults.

CHOLESTERYL ESTER transfer protein (CETP) is a hydrophobic glycoprotein that mediates the net transfer of neutral lipids between lipoproteins by stimulating the heteroexchange of cholesteryl esters and triglycerides.[1,2] It redistributes cholesteryl esters formed by lecithin:cholesterol acyltransferase in high-density lipoprotein (HDL) to the less dense apolipoprotein B (apo B)–containing lipoproteins. Therefore, it plays an important role in the metabolism of HDL and apo A-I and in the reverse cholesterol transport pathway.[1-3]

In viewing 4 published reports,[3-6] thyroid hormones seem to be involved in the regulation of CETP, and CETP may play a role in the alterations of HDL lipids observed in hypothyroidism. According to these studies,[3-6] plasma CETP activity was low in hypothyroid adults, and a significant increase in plasma CETP activity was noted after thyroxine replacement. However, as we discuss later in this report, the observed differences in plasma CETP activity were small and some other factors may have contributed to their results. Although the reported results may seem to explain the mechanisms of the elevation of HDL cholesterol in hypothyroid states, we think it is too early to draw any conclusions based on the limited data obtained only from adult patients.

In adults, the presence of a multitude of environmental determinants, including smoking,[7,8] drinking,[9-11] exercise,[12] estrogen use,[13] diabetes mellitus,[14] and obesity,[15,16] that can affect CETP may make the assessment difficult. Early in infancy, when the effects of many of the above-mentioned factors have not yet been established, hormonal influences are easier to quantify. The aim of the present study was to investigate the effects of thyroid dysfunction on CETP by studying plasma CETP activity in hypothyroid infants before and after they were rendered euthyroid with L-thyroxine (LT_4) replacement therapy.

From the Department of Pediatrics, School of Medicine, Niigata University, Niigata, Japan.

Submitted October 18, 1999; accepted March 24, 2000.

Address reprint requests to Tadashi Asami, MD, Department of Pediatrics, School of Medicine, Niigata University, Asahimachi-dori 1-757, Niigata, Japan 951-8510.

0026-0495/00/4909-0017$10.00/0
doi:10.1053/meta.2000.8617

SUBJECTS AND METHODS

Fourteen hypothyroid infants (aged 1 month; 7 males and 7 females) before LT_4 replacement therapy, 23 normal infants (aged 1 month; 11 males and 12 females), and 61 normal adults (aged 23 to 54 years; 38 males and 23 females) were enrolled in this study. The 14 hypothyroid infants were identified during neonatal mass screening for congenital hypothyroidism. These patients had elevated blood thyrotropin (TSH) levels (>97th percentile of the assay) and were referred to our hospital for further evaluation at the age of 1 month. The 14 infants were later confirmed to have congenital hypothyroidism. The 23 normal infants were selected from neonates who were referred to our hospital because of transiently elevated blood methionine, leucine, or galactose during newborn mass screening. TSH levels in their dried blood samples measured during the screening were all completely within the normal range, and their suspected disorders were finally ruled out.

Blood samples were obtained for measurement of plasma CETP activity according to the method of Kato et al,[17] and serum TSH, free triiodothyronine (FT_3), free thyroxine (FT_4), and other parameters were also measured on an autoanalyzer (Hitachi 7170). Informed consent was obtained from the parents when they first visited our hospital. Plasma CETP activity was analyzed by measuring the transfer of cholesteryl esters from exogenous radiolabeled HDL to apo B–containing lipoproteins.

At the age of 1 month, the first blood samples were taken and serum thyroid hormone levels of the 14 infants indicated a hypothyroid status: TSH 306.9 ± 109.9 μIU/mL (normal range, 0.6 to 5.3), FT_4 0.77 ± 0.21 ng/dL (normal range, 1.3 to 2.8), and FT_3 2.97 ± 0.35 pg/mL (normal range, 2.1 to 3.8). The next day, LT_4 substitution therapy was started. Each patient received an oral dose of LT_4 approximately 10 μg/kg body weight once per day in the morning. Two months after LT_4 substitution, at the age of 3 months, the second blood samples were taken and serum thyroid hormone levels were confirmed as normalized: TSH 3.24 ± 0.71 μIU/mL, FT_4 2.67 ± 0.24 ng/dL, and FT_3 3.90 ± 0.25 pg/mL.

An additional 18 euthyroid infants aged between 3 and 11 months, who were rendered euthyroid by LT_4 replacement since 1 month of age, were enrolled as the subjects in this study to assess the relationships of CETP with thyroid hormones.

Measurement of CETP Activity

Plasma CETP activity was measured according to the method of Kato et al.[17] Briefly, discoidal bilayer particles were used as cholesteryl ester donors and LDL was used as the acceptor. The LDL used in this study was separated from pooled plasma obtained from normal adults with a normal lipid profile. The transfer of ^{14}C-labeled cholesteryl oleate in discoidal bilayer particles to LDL was monitored after incubation for 30 minutes at 37°C with or without the addition of 2 μL plasma as the source of CETP. Discoidal bilayer particles and LDL were separated by sodium dextran sulfate and $MgCl_2$ dextran sulfate and $MgCl_2$ precipitation, and radioactivity in the supernatant and precipitates was then determined. CETP activity linearly increased within 30 minutes of incubation. We expressed CETP activity as a percent of the control value.[17,18] The control value for plasma CETP activity was obtained from 47 normal adults working in the R & D Center Biomedical Laboratories (BML) (Saitama, Japan). The 47 subjects were selected because they had a normal serum lipid profile and no D442G or intron 14 mutations in the human CETP gene. The 47 samples were measured for the cholesteryl ester transfer rate, and the mean transfer rate was used as the control value for plasma CETP activity. Three plasma samples, the transfer rates of which were close to the mean value, were separated and kept frozen at −80°C to be used as the control value in each assay. All of these laboratory procedures were performed by the BML (Saitama, Japan).

Statistical Analysis

Since plasma CETP activity and thyroid hormone concentrations were not normally distributed, the significance of the difference in these values was analyzed by nonparametric *t* test. The significance of the values before and after LT_4 therapy was determined by Wilcoxon's signed rank sum test. Data are presented as the mean ± SD. Linear regression was used to assess the correlation of plasma CETP activity with thyroid hormones. A *P* value less than .05 was considered significant.

RESULTS

Plasma CETP Activity

Plasma CETP activity was not statistically different in hypothyroid infants and normal infants. Both hypothyroid and normal infants had significantly higher plasma CETP activity than normal adults (Fig 1).

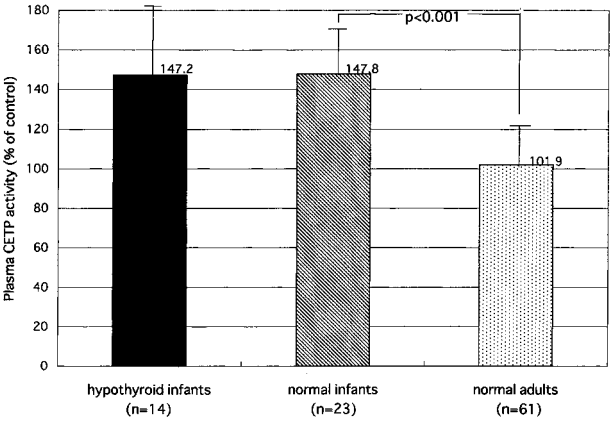

Fig 1. Plasma CETP activity. Plasma CETP activity was not statistically different in hypothyroid infants (n = 14, 147.2% ± 32.8% of control) and normal infants (n = 23, 147.8% ± 21.7% of control). Normal infants had significantly higher plasma CETP activity than normal adults (n = 61, 101.9% ± 19.8%, *P* < .001).

Changes in Plasma CETP Activity Before and After LT_4 Replacement

In hypothyroid infants, serum thyroid hormone levels were normalized 2 months after LT_4 replacement (at the age of 3 months). However, no significant changes were observed in plasma CETP activity after LT_4 replacement (Fig 2).

Relationship of Plasma CETP Activity With Thyroid Hormones

In the 14 hypothyroid infants before LT_4 substitution, plasma CETP activity did not show any relationship with thyroid hormone levels. In the 32 euthyroid infants during LT_4 replacement, there was no relationship between plasma CETP activity and thyroid hormone levels (Table 1).

DISCUSSION

In this study, we found no differences in plasma CETP activity between hypothyroid infants and age-matched normal infants. LT_4 substitution did not cause any changes in plasma CETP activity after therapy. Plasma CETP activity showed no correlation with serum TSH, FT_4, and FT_3 levels. Both hypothyroid and normal infants showed higher plasma CETP activity than normal adults.

CETP activity may be regulated in 4 ways[2]: by factors that influence the concentration of CETP in plasma, by the activity of CETP inhibitor proteins, by variations in the concentration and composition of donor and acceptor lipoproteins, and by factors that influence the interaction of CETP with plasma lipoproteins. Considering the presence of high serum HDL cholesterol and apo A-I levels in hypothyroid patients,[19-24] thyroid hormones seem to be one of the factors affecting plasma CETP activity.

In viewing the literature, there have been 4 reports on the relationship between plasma CETP and thyroid hormones. Dullaart et al[4] first investigated the effect of hypothyroidism on CETP activity in 13 athyrotic patients before and after triiodothyronine (T_3) supplementation for 33 to 67 days. According to

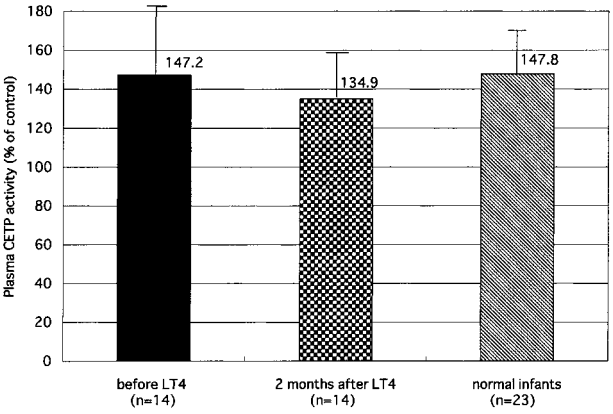

Fig 2. Changes in plasma CETP activity before and after LT_4 replacement. No significant changes were observed in plasma CETP activity after LT_4 replacement: 14 hypothyroid infants before LT_4, 147.2% ± 32.8% of control; 2 months after LT_4, 134.9% ± 23.4% of control; and 23 normal infants, 147.8% ± 21.7% of control.

Table 1. Correlation Coefficients of Plasma CETP Activity Versus Thyroid Hormone Levels in Hypothyroid and Treated Infants

Hormone	Before LT_4 Replacement (n = 14)	After LT_4 Replacement (n = 32)
TSH	−.001	.208
FT_4	−.237	.091
FT_3	.001	.187

NOTE. All values are statistically nonsignificant.

their study, CETP activity was 15% lower in the hypothyroid state as compared with the euthyroid state, after T_3 supplementation.[4] However, the difference was very small and the duration of T_3 supplementation varied widely from 33 days to 67 days, which may have affected the results. In our study, we measured all variables exactly 2 months after the start of LT_4 replacement, and the ages of the patients were all the same. Another point to consider in their study is that there were no differences in plasma CETP activity in their 13 hypothyroid patients versus 26 euthyroid controls. This result is very similar to the present study, suggesting that there are no relationships between plasma CETP and thyroid hormone levels.

In another study by Ritter et al,[5] plasma CETP activity was significantly decreased when patients were hypothyroid and increased to normal levels after hormone replacement and restoration of eumetabolism. However, plasma CETP concentrations were unchanged before and after treatment in hypothyroid females.[5] Based on their observations, they concluded that decreases in CET during hypothyroidism may be secondary to acceptor lipoprotein (LDL and very–low-density lipoprotein) changes in the hypothyroid state and not to changes in the concentration of CETP itself.[5] Their report seems to partially support our views.

In more recent studies by Tan et al,[3] plasma CETP activity was increased in hyperthyroid patients compared with controls, whereas in hypothyroid patients, plasma CETP activity was decreased. In the hypothyroid patients, there was a significant increase in plasma CETP activity after thyroxine replacement. In addition, there was a strong positive correlation between logarithmic (FT_4) and plasma CETP activity.[3] However, we did not find any relationships between plasma CETP activity and thyroid hormones not only in hypothyroid infants before LT_4 substitution but also in a larger number of euthyroid infants receiving long-term LT_4 replacement therapy since 1 month of age.

With regard to the response of CETP to thyroid hormone replacement, although Tan et al[3] obtained a statistically significant increase in plasma CETP activity after LT_4 replacement in hypothyroid patients, the mean increase was very small (4.32%) and the 2 patients, showing prominent increases in plasma CETP activity, could have contributed to the statistical significance of their results. Another point is that no significant changes were observed in plasma CETP activity in hyperthyroid patients before and after therapy in another one of their studies.[6] In our study, there were no changes in plasma CETP activity exactly 2 months after LT_4 replacement therapy with complete normalization of thyroid hormone levels.

As described before, the results of the present study are very different from those in the 4 previous studies wherein plasma CETP activity was low in the hypothyroid state. In contrast to these 4 previous studies, we found no differences in plasma CETP between hypothyroid and normal infants and no changes in plasma CETP before and after LT_4 replacement. In our study, plasma CETP activities were neither affected by thyroid status nor correlated with thyroid hormone levels.

Although there seems to be no obvious explanation for this difference, one possible explanation might be that in the previous studies all subjects were adults, whose plasma CETP activity tends to be affected by many environmental factors. In clinical studies enrolling adult patients as subjects, it can be difficult to adjust the obtained values for all of these environmental factors, which may then affect the results. To minimize the possible effects of such factors on thyroid hormones and CETP, we elected to study infants who had not yet been exposed to these factors.

Another possible explanation for the different results of our study versus the others would be that adults may respond to thyroid hormone replacement very differently from infants. According to a report by Tenenbaum et al,[25] in contrast to adult hypothyroidism, LDL catabolism is not altered in congenital hypothyroidism in infants. However, as in adults, a defect of lipolytic enzyme activities is present and can induce an impairment of the elimination of cholesterol via the HDL route and potentially of the catabolism of triglyceride-rich lipoproteins.[25] Another support for this possible age difference in lipid metabolism is that in our study both hypothyroid and normal infants were found for the first time to have very high plasma CETP activity compared with normal adults. The significance of the observed high plasma CETP activity in infants remains uncertain on the basis of the available data.

Based on our findings, we conclude that in infants thyroid hormones do not affect plasma CETP activity, and normal infants have high plasma CETP activity compared with normal adults. Further studies from other institutions are needed to confirm this.

REFERENCES

1. Tall AR: Plasma cholesteryl ester transfer protein. J Lipid Res 34:1255-1274, 1993

2. Barter P, Rye KA: Cholesteryl ester transfer protein: Its role in plasma lipid transport. Clin Exp Pharmacol Physiol 21:663-672, 1994

3. Tan KC, Shiu SW, Kung AW: Plasma cholesteryl ester transfer protein (CETP) activity in hyper- and hypothyroidism. J Clin Endocrinol Metab 83:140-143, 1998

4. Dullaart RP, Hoogenberg K, Groener JE, et al: The activity of cholesteryl ester transfer protein is decreased in hypothyroidism: A possible contribution to alterations in high-density lipoproteins. Eur J Clin Invest 20:581-587, 1990

5. Ritter MC, Kannan CR, Bagdade JD: The effects of hypothyroidism and replacement therapy on cholesteryl ester transfer. J Clin Endocrinol Metab 81:797-800, 1996

6. Tan KC, Shiu SW, Kung AW: Effect of thyroid dysfunction on high-density lipoprotein subfraction metabolism: Roles of hepatic

lipase and cholesteryl ester transfer protein (CETP). J Clin Endocrinol Metab 83:2921-2924, 1998

7. Dullaart RP, Hoogenberg K, Dikkeschei BD, et al: Higher plasma lipid transfer protein activities and unfavorable lipoprotein changes in cigarette-smoking men. Arterioscler Thromb 14:1581-1585, 1994

8. Mero N, Van Tol A, Scheek LM, et al: Decreased postprandial high density lipoprotein cholesterol and apolipoproteins A-I and E in normolipidemic smoking men: Relations with lipid transfer proteins and LCAT activities. J Lipid Res 39:1493-1502, 1998

9. Valimaki M, Kahri J, Laitinen K, et al: High density lipoprotein subfractions, apolipoprotein A-I containing lipoproteins, lipoprotein(a), and cholesterol ester transfer protein activity in alcoholic women before and after ethanol withdrawal. Eur J Clin Invest 23:406-417, 1993

10. Hannuksela ML, Liinamaa MJ, Kesaniemi YA, et al: Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. Atherosclerosis 110:35-44, 1994

11. Liinamaa MJ, Kesaniemi YA, Savolainen MJ: Lipoprotein composition influences cholesteryl ester transfer in alcohol abusers. Ann Med 30:316-322, 1998

12. Foger B, Wohlfarter T, Ritsch A, et al: Kinetics of lipids, apolipoproteins, and cholesteryl ester transfer protein in plasma after a bicycle marathon. Metabolism 43:633-639, 1994

13. Vadlamudi S, MacLean P, Green T, et al: Role of female sex steroids in regulating cholesteryl ester transfer protein in transgenic mice. Metabolism 47:1048-1051, 1998

14. Kahri J, Groop PH, Elliott T, et al: Plasma cholesteryl ester transfer protein and its relationship to plasma lipoproteins and apolipoprotein A-I–containing lipoproteins in IDDM patients with microalbuminuria and clinical nephropathy. Diabetes Care 17:412-419, 1994

15. Arai T, Yamashita S, Hirano K, et al: Increased plasma cholesteryl ester transfer protein in obese subjects. A possible mechanism for the reduction of serum HDL cholesterol levels in obesity. Arterioscler Thromb 14:1129-1136, 1994

16. Dullaart RP, Sluiter WJ, Dikkeschei LD, et al: Effect of adiposity on plasma lipid transfer protein activities: A possible link between insulin resistance and high density lipoprotein metabolism. Eur J Clin Invest 24:188-194, 1994

17. Kato H, Nakanishi T, Arai H, et al: Purification microheterogeneity, and stability of human lipid transfer protein. J Biol Chem 264:4082-4087, 1989

18. Arai T, Yamashita S, Sakai N, et al: A novel nonsense mutation (G181X) in the human cholesteryl ester transfer protein gene in Japanese hyperalphalipoproteinemic subjects. J Lipid Res 37:2145-2154, 1996

19. Raziel A, Rosenzweigh B, Borvinic V, et al: The influence of thyroid function on serum lipid profile. Atherosclerosis 41:321-326, 1982

20. Aviram M, Luboshitzky R, Brook J: Lipid and lipoprotein pattern in thyroid dysfunction and the effect of therapy. Clin Biochem 15:62-66, 1982

21. Wiseman SA, Powell JT, Humphries SE, et al: The magnitude of the hypercholesterolemia of hypothyroidism is associated with variation in the low density lipoprotein receptor gene. J Clin Endocrinol Metab 77:108-112, 1993

22. Spandrio S, Sleiman I, Scalvini T, et al: Lipoprotein(a) in thyroid dysfunction before and after treatment. Horm Metab Res 25:586-589, 1993

23. Asami T, Kikuchi T, Uchiyama M: Effects of L-thyroxine on serum lipid profiles in infants with congenital hypothyroidism. J Pediatr 127:812-814, 1995

24. Asami T, Ciomartan T, Uchiyama M: Changes in serum apolipoprotein concentrations after L-thyroxine therapy in infants with congenital hypothyroidism. Metabolism 48:1343-1345, 1999

25. Tenenbaum D, Gambert P, Fischbach M, et al: Alterations of serum high-density lipoproteins and hepatic lipase activity in congenital hypothyroidism. Biol Neonate 54:241-245, 1988