

ORIGINAL

Effect of L-thyroxine replacement on apolipoprotein B-48 in overt and subclinical hypothyroid patients

Mitsuru Ito¹⁾, Akira Kitanaka²⁾, Takeshi Arishima¹⁾, Takumi Kudo¹⁾, Eijun Nishihara¹⁾, Sumihisa Kubota¹⁾, Nobuyuki Amino¹⁾, Tetsuya Hiraiwa³⁾, Toshiaki Hanafusa³⁾ and Akira Miyauchi¹⁾

¹⁾ Kuma Hospital, Kobe 650-0011, Japan

²⁾ Department of Internal Medicine, Faculty of Medicine, Kagawa University, Kita-gun 761-0793, Japan

³⁾ First Department of Medicine, Osaka Medical College, Takatsuki 569-8686, Japan

Abstract. Apolipoprotein B-48 (ApoB-48) is a constituent of chylomicrons and chylomicron remnants, and is thought to be one of the risk factors for atherosclerosis. We evaluated the effect of L-thyroxine (L-T₄) replacement on serum ApoB-48 levels in patients with primary hypothyroidism. Eighteen patients with overt hypothyroidism (OH) and 18 patients with subclinical hypothyroidism (SH) participated in the study. The lipid profiles, including ApoB-48, were measured in patients with hypothyroidism before and 3 months after L-T₄ replacement. After L-T₄ replacement, the serum concentrations of all lipoproteins, exclusive of lipoprotein(a) (Lp(a)), were significantly decreased in patients with OH. In patients with SH, the serum levels of total cholesterol (TC), non-high-density lipoprotein cholesterol (non-HDL-C), remnant-like particle cholesterol (RLP-C), apolipoprotein B (ApoB), and ApoB-48 decreased significantly after L-T₄ replacement. The serum levels of triglycerides (TG), HDL-C, low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (ApoA-1), and Lp(a) did not change significantly. In all 36 patients, the reduction in the ApoB-48 levels correlated significantly with the reduction in TSH levels ($r = 0.39$, $P < 0.05$). This study showed clearly that L-T₄ replacement might reduce serum levels of ApoB-48 in both OH and SH patients. Such altered serum levels of ApoB-48 in patients with OH and SH may be related to the disturbed metabolism of chylomicron remnants in patients with hypothyroidism.

Key words: Apolipoprotein B-48, Chylomicron remnant, Hypothyroidism, L-thyroxine, Lipoprotein

DYSLIPIDEMIA is related to the pathogenesis of atherosclerosis. The relationship between cardiovascular disease (CVD) and hypercholesterolemia, increased low-density lipoprotein cholesterol (LDL-C), or decreased high-density lipoprotein cholesterol (HDL-C) has been well documented.

Dietary fats are transported as chylomicrons, which are macromolecules synthesized exclusively by the intestine. Chylomicrons when first secreted are triglyceride (TG) rich; however, once in circulation, they rapidly undergo hydrolysis to produce cholesterol-enriched remnants. It is the TG-depleted remnants that are considered to be atherogenic, because they are able to penetrate arterial tissue and become trapped within the sub-

endothelial space. Indeed, chylomicron remnants can induce substantial macrophage lipid loading, a hallmark feature of early atherogenesis [1, 2].

Several tests are used to assess TG-rich lipoprotein kinetics. Elevated fasting plasma TG level and postprandial response have been widely used as markers for the metabolism of chylomicrons. Alternatively, chylomicron remnant metabolism is often studied after an oral fat load containing esterified vitamin A [3]. Chylomicrons and chylomicron remnants have a characteristic apolipoprotein, apolipoprotein B-48 (ApoB-48), with each having one ApoB-48 molecule per particle. Hence, ApoB-48 could be a good indicator for chylomicrons and chylomicron remnants derived from the intestine [4]. Some investigators have reported that serum ApoB-48 levels were increased in familial hyperlipidemia [5, 6], type 2 diabetes [7], and metabolic syndrome [8].

Hypothyroidism is associated with an atherogenic

Submitted Jun. 20, 2012; Accepted Aug. 25, 2012 as EJ12-0226
Released online in J-STAGE as advance publication Sep. 15, 2012
Correspondence to: Mitsuru Ito, M.D., Kuma Hospital, 8-2-35, Shimoyamate-Dori, Chuo-Ku, Kobe 650-0011, Japan.
E-mail: ito02@kuma-h.or.jp

lipid profile that includes greater serum concentrations of LDL-C [9, 10] and apolipoprotein(a) (Lp(a)) [11]. With regard to the TG-rich lipoprotein in hypothyroidism, we previously reported the disturbed metabolism of remnant lipoprotein in overt hypothyroidism (OH) [12] and subclinical hypothyroidism (SH) [13].

In the present study, we measured the serum concentrations of ApoB-48 in patients with OH and SH before and after T₄ replacement to investigate the effect of thyroid hormone replacement on ApoB-48 concentration, a good indicator of chylomicron remnants derived from the intestine, in patients with hypothyroidism.

Materials and Methods

Patients

We recruited 18 patients with OH (mean age, 54±9 years; mean body mass index, 24.8±4.8 kg/m²) and 18 patients with SH (mean age, 58±7 years; mean body mass index, 21.8±3 kg/m²) who had been referred to Kuma Hospital in Kobe, Japan. OH was diagnosed on the basis of elevated serum TSH levels and lowered free thyroxine (free T₄) levels. The causes of OH included Hashimoto thyroiditis (n = 17) and radioiodine therapy (n = 1) for hyperthyroidism. SH was diagnosed on the basis of elevated serum TSH levels (≥6 μIU/mL) and free thyroid hormone levels (free T₄ and free T₃) within the normal range. The onset of SH in each patient was well established, as the patients had been followed for several months, beginning when the increase in TSH level was detected. The causes of SH included Hashimoto thyroiditis (n = 17) and radioiodine therapy (n = 1) for hyperthyroidism. None of the patients had a history of coronary heart disease, acute illness, or disorders that affect lipid metabolism (e.g., diabetes mellitus, renal failure, nephrotic syndrome, or pancreatitis). None of the patients were on a lipid-lowering agent during the study period. All patients gave their informed consent for participation in the study, which was approved by the Institutional Ethics Committee.

Study protocol

After both the patients with OH and the patients with SH fasted overnight, blood samples were drawn to determine the serum lipid concentrations and thyroid function test results at the baseline. L-T₄ replacement was then initiated (25 or 50 μg/day) in the patients. All patients were advised to maintain their dietary habits

during the study period. To normalize the serum TSH levels, the L-T₄ dosage was adjusted according to the serum free T₄ and TSH concentrations measured at 4-week intervals after L-T₄ replacement was initiated. The mean final dose of L-thyroxine required to normalize the serum TSH levels was 99 ± 29 μg/day in the OH patients and 60 ± 13 μg/day in the SH patients. In the patients treated with L-T₄, the lipid profiles were evaluated after 3 months.

Laboratory determinations

The levels of total cholesterol (TC), HDL-C, and TG were measured by enzyme assays. The non-HDL-C levels were calculated as [TC – HDL-C]. The LDL-C levels were calculated by the Friedewald formula. The serum Lp(a) concentration was measured using a latex agglutination assay (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Apolipoprotein B (ApoB), Apolipoprotein E (ApoE), and apolipoprotein A1 (ApoA-1) were measured by immunoturbidimetry. The remnant-like particle cholesterol (RLP-C) was prepared using an immunoseparation technique (Japan Immunoresearch Laboratories, Takasaki, Japan) [14]. Apolipoprotein B-48 (ApoB-48) was measured by the ELISA method using a monoclonal antibody [15]. The intraassay coefficient of variation (CV) for serum ApoB-48 ranged from 2.3–4.4 %. The normal ranges were 3.06 – 6.14 μg/mL for ApoB-48. Serum concentrations of TSH, FT₄, and FT₃ were measured with a chemiluminescent immunoassay (ARCHITECT i2000; Abbott Japan, Tokyo, Japan). The normal ranges were 0.3 – 5.0 μIU/mL for TSH, 0.7–1.6 ng/dL for FT₄, and 1.7 – 3.7 pg/mL for FT₃.

Statistical analysis

Grouped data were expressed as means±SD. Treatment effects (pre- versus post-L-thyroxine- T₄ (L-T₄) replacement) were analyzed using a paired *t*-test for normal distribution and using the Wilcoxon signed rank test for nonparametric distribution. Significance was defined as a corresponding *P* value of less than 0.05 (two-sided). Pearson's correlation coefficient test was used to assess the correlation between the reductions in TSH, ApoB-48, and other variables.

Results

The characteristics and lipid profiles of patients with OH and SH before and after L-T₄ replacement

are listed in Table 1. Before correction of the hypothyroid state, the mean TSH level was 78.7 ± 26 μ IU/mL in patients with OH and 9.34 ± 4.37 μ IU/mL in patients with SH. After L-T₄ replacement, the serum levels of TSH and free T₄ were within the normal range in all patients. Body mass indices were unchanged in both OH and SH.

In the patients with OH, the levels of serum TC, HDL-C, non-HDL-C, LDL-C, RLP-C, TG, ApoB, and ApoA-1 were significantly decreased after L-T₄ replace-

ment. The serum Lp(a) levels were unchanged. In patients with SH, the serum levels of TC, non-HDL-C, RLP-C, and ApoB decreased significantly after L-T₄ replacement. The serum levels of TG, HDL-C, LDL-C, ApoA-1, and Lp(a) did not change significantly.

In both the patients with OH and those with SH, the levels of serum ApoB-48 were significantly decreased after L-T₄ replacement ($P < 0.005$, $P < 0.05$, respectively) (Table 1). Fig. 1 shows the changes in the serum concentrations of ApoB-48 before and after L-T₄ replace-

Table 1 The characteristics and lipid profiles in patients with overt and subclinical hypothyroidism before and after L-T₄ replacement therapy

	Overt hypothyroidism (n=18)		Subclinical hypothyroidism (n=18)	
	Before	After	Before	After
BMI (kg/m ²)	24.8 \pm 4.8	24.4 \pm 5.2	21.8 \pm 3.0	21.6 \pm 2.9
TSH (μ IU/mL)	78.7 \pm 26	2.78 \pm 2.13 ^a	9.34 \pm 4.37	2.09 \pm 1.82 ^a
FT ₄ (ng/dL)	0.33 \pm 0.19	1.03 \pm 0.17 ^a	0.91 \pm 0.17	1.24 \pm 0.28 ^a
FT ₃ (pg/mL)	1.68 \pm 0.55	2.50 \pm 0.45 ^a	2.84 \pm 0.42	2.93 \pm 0.59
TC (mg/dL)	267 \pm 57	205 \pm 34 ^a	243 \pm 35	229 \pm 36 ^a
TG (mg/dL)	124 \pm 45	90 \pm 35 ^a	120 \pm 48	105 \pm 30
HDL-C (mg/dL)	77 \pm 17	64 \pm 17 ^a	68 \pm 17	64 \pm 17 [*]
LDL-C (mg/dL)	166 \pm 48	123 \pm 30 ^a	152 \pm 30	144 \pm 29
Non-HDL-C (mg/dL)	190 \pm 52	141 \pm 35 ^a	175 \pm 31	165 \pm 31 ^a
ApoB (mg/dL)	121 \pm 36	92 \pm 23 ^a	116 \pm 21	109 \pm 20 ^a
ApoA-1 (mg/dL)	170 \pm 24	152 \pm 29 ^a	160 \pm 29	152 \pm 32
Lp (a) (mg/dL)	16 \pm 15	14 \pm 13	17 \pm 16	15 \pm 13 [*]
RLP-C (mg/dL)	6.1 \pm 2.1	3.9 \pm 1.4 ^a	6.0 \pm 2.4	4.9 \pm 1.6 ^a
ApoB-48 (μ g/mL)	22.6 \pm 21.3	7.9 \pm 9.2 ^a	7.6 \pm 9.3	3.7 \pm 3.7 ^a

Values are the mean \pm SD. Replacement effects of L-T₄ were analyzed by paired *t* test and by ^{*}Wilcoxon signed rank test for nonparametric distribution. ^a $P < 0.05$ vs. before L-T₄ replacement therapy

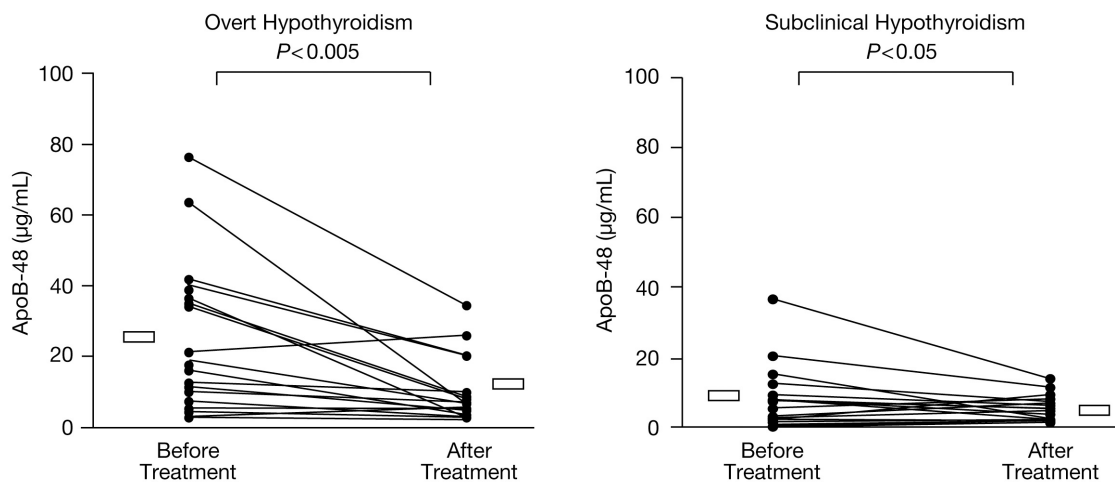


Fig. 1 Individual changes in the serum levels of apolipoprotein B-48 before and after L-thyroxine replacement in patients with overt hypothyroidism and subclinical hypothyroidism. Open squares represent means.

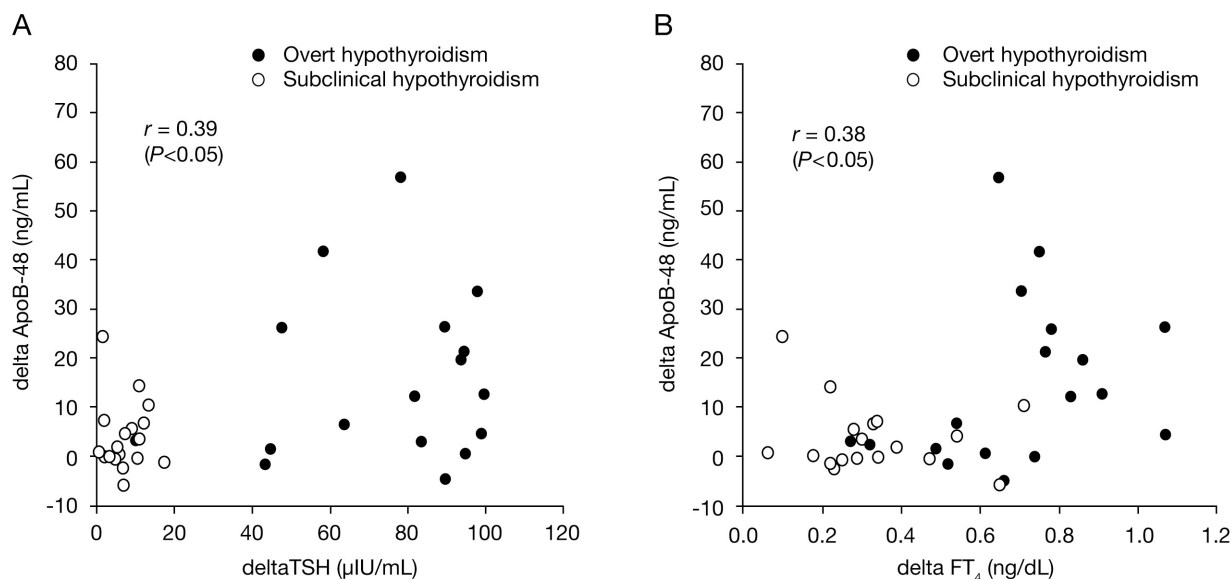


Fig. 2 Relation between treatment-induced reductions in levels of thyroid-stimulating hormone (TSH) (A) and free T₄ (FT₄) (B) and apolipoprotein B-48 in patients with hypothyroidism
Closed circles represent overt hypothyroid patients. Open circles represent subclinical hypothyroid patients.

ment in patients with OH and SH. Sixteen of 18 patients with OH responded to L-T₄ replacement with a decrease in serum Apo-48. On the other hand, 12 of 18 patients with SH responded to L-T₄ replacement with a decrease in serum Apo-48.

Fig. 2 presents the correlation coefficients between the reduction in TSH and the elevation in FT₄ levels and the reduction in the ApoB-48 levels in all 36 patients. The reduction in the ApoB-48 levels correlated significantly with the reduction in TSH levels ($r = 0.39$, $P < 0.05$) and the elevation in FT₄ levels ($r = 0.38$, $P < 0.05$). On the other hand, the reduction in the ApoB-48 levels and those in the TSH were not significant ($r = 0.24$, $P = 0.112$) in only SH patients.

The correlations of reductions with L-T₄ replacement in serum ApoB-48, RLP-C, TG, and LDL-C were evaluated. The reduction in the ApoB-48 levels and those in the RLP-C ($r = 0.26$, $P = 0.123$) were not significant. The only significant association was found between the reduction in the RLP-C and the reduction in TG ($r = 0.72$, $P < 0.0001$).

Discussion

We have demonstrated that L-T₄ replacement induces a reduction of fasting serum levels of ApoB-48, which is a static marker of chylomicrons and chylomicron

remnants, in patients with OH and SH. In addition, the reduction in ApoB-48 levels correlated significantly with the reduction in TSH levels and the elevation in FT₄ levels.

This change was accompanied by reductions in serum levels of TC, non-HDL-C, RLP-C, and ApoB, and no change in Lp(a). These accompanied data were compatible with those of our previous report [16].

Recently, Mugii *et al.* evaluated the correlations between serum ApoB-48 and thyroid hormone in patients with various thyroid states, and reported that serum ApoB-48 concentrations were significantly higher in OH and SH subjects compared to those in normal subjects [17]. They also evaluated the effect of L-T₄ replacement on the serum ApoB-48 concentration in OH patients. However, there were differences between our study and theirs in the treatment periods of L-T₄ and the number of patients. The treatment periods in the present study was three months, while their treatment period was only one month. The present study included 18 patients, while Mugii *et al.* evaluated 5 patients, and only indicated the data from two patients. Moreover, they did not indicate the effect of L-T₄ replacement on the serum ApoB-48 concentration in SH patients.

In regard to metabolism of chylomicrons in hypothyroidism, Abrams *et al.* reported no abnormality in

patients with hypothyroidism [18], while other investigators showed significantly decreased clearance of chylomicron remnants in hypothyroid rats [19, 20]. Weintraub *et al.* reported that postprandial accumulation of chylomicron remnants exists in the hypothyroid state as a way of indicating an absorption disorder of vitamin A [21]. However, such a method has some limitations, and one of them is that the metabolism of vitamin A may be disturbed in subjects with hypothyroidism [22]. Additionally, this method requires fat-loading with vitamin A and monitoring of a long time course. In this study, we measured fasting serum levels of ApoB-48, which is a more quantitative and direct method. It has been reported that a high fasting ApoB-48 level reflects high postprandial levels of chylomicrons and/or chylomicron remnants [23, 24]; therefore, we assumed that a high fasting serum level of ApoB-48 indicated the existence of postprandial hyperlipidemia associated with chylomicrons and/or chylomicron remnants. Our results are in agreement with the findings of animal studies and indirect human study in hypothyroidism. These results may be explained in part by findings in previous studies. LPL and HTGL, which are essential for the degradation of chylomicrons or chylomicron remnants, were demonstrated to significantly increase after T₄ replacement [10, 12]. Hepatocyte B-E receptors that are responsible for the uptake of LDL and chylomicrons were depressed in subjects with hypothyroidism, with an increase in expression in response to the administration of thyroid hormone [25, 26].

Because our subjects with hypothyroidism showed higher serum levels of ApoB-48, it is possible that chylomicron remnants play a role in the increased risk of atherosclerosis related to hypothyroidism. Proctor *et al.* reported intimal retention of cholesterol derived from ApoB-48-containing lipoproteins in the carotid arteries of hyperlipidemic rabbits [27]. Several clinical studies indicated that serum ApoB-48 level was associated with atherosclerosis in humans [28, 29]. Whether the serum ApoB-48 level in hypothyroidism influences atherosclerosis remains to be determined.

Both ApoB-48 and RLP-C were used as markers of TG-rich lipoprotein; however, the reduction in the ApoB-48 levels with L-T₄ replacement was not correlated to the reduction in RLP-C levels in the present study. One possible explanation is that the change in the cholesterol content of chylomicron remnants (reflected by RLP-C) with L-T₄ replacement did not

differ from the change in remnant particle number (reflected by ApoB-48). Another possibility is that the reduction in the RLP-C levels reflects a reduction in the levels of hepatic lipoproteins, because RLP-C reflects a lipoprotein population that is both intestinally (chylomicron remnants) and hepatically derived (VLDL remnants) [30]. On the other hand, ApoB-48 mainly reflects chylomicrons and chylomicron remnants derived from the intestine. Indeed, several previous studies reported that there is a discrepancy in serum basal level or in treatment effect between ApoB-48 and RLP-C in patients with hyperlipidemia [5, 31]. These findings may explain the lack of correlation between the reduction effect of L-T₄ replacement in ApoB-48 and that in RLP-C in hypothyroid patients.

OH, with its accompanying hypercholesterolemia, is widely recognized as a risk factor for atherosclerosis and cardiovascular disease [32]. Necropsy studies confirmed an association between OH and coronary heart disease [33, 34]. On the other hand, although SH is highly prevalent, it is controversial whether SH is a risk factor for cardiovascular disease. Some previous studies [35, 36] suggested that SH indicated a risk for cardiovascular disease, but others suggested that it did not [37]. Whether there is an association between ApoB-48 levels and cardiovascular disease in patients with OH and SH remains to be determined.

There were some possible limitations in the present study. First, the study was not a placebo-controlled design. In addition, in the present study, the reduction in the ApoB-48 levels and those in the TSH were not significant in only SH patients. We may not observed a significant correlation because of our relatively small sample or short-term (3 months) treatment period, or because our SH patients had relatively mild increases in serum TSH levels (mean, 9.34 μ IU/mL). Properly controlled studies are needed to demonstrate whether T₄ replacement therapy alters ApoB-48 levels in patients with hypothyroidism.

In summary, the present study demonstrated, probably for the first time, that L-T₄ replacement induces a reduction of serum concentrations of ApoB-48 in both OH and SH. Our results also suggest that L-thyroxine replacement may have beneficial effects on the metabolism of ApoB-48 in addition to the already known LDL-C and ApoB, which may be relevant for reducing the risk of cardiovascular complications in SH.

References

- Mamo JC, Yu KC, Elsegood CL, Smith D, Vine D, et al. (1997) Is atherosclerosis exclusively a postprandial phenomenon? *Clin Exp Pharmacol Physiol* 24:288-293.
- Gianturco SH, Brown FB, Gotto AM Jr, Bradley WA (1982) Receptor-mediated uptake of hypertriglyceridemic very low density lipoproteins by normal human fibroblasts. *J Lipid Res* 23:984-993.
- Berr F, Eckel R, Kern F Jr (1985) Plasma decay of chylomicron remnants is not affected by heparin-stimulated plasma lipolytic activity in normal fasting man. *J Lipid Res* 26:852-859.
- Phillips ML, Pullinger C, Kroes I, Kroes J, Hardman DA, et al. (1997) A single copy of apolipoprotein B-48 is present on the human chylomicron remnant. *J Lipid Res* 38:1170-1177.
- Dane-Stewart CA, Watts GF, Mamo JC, Dimmitt SB, Barrett PH, et al. (2001) Elevated apolipoprotein B-48 and remnant-like particle-cholesterol in heterozygous familial hypercholesterolaemia. *Eur J Clin Invest* 31:113-117.
- Verseyden C, Meijssen S, Cabezas MC (2004) Effects of atorvastatin on fasting plasma and marginated apolipoproteins B48 and B100 in large, triglyceride-rich lipoproteins in familial combined hyperlipidemia. *J Clin Endocrinol Metab* 89:5021-5029.
- Hogue JC, Lamarche B, Tremblay AJ, Bergeron J, Gagné C, et al. (2007) Evidence of increased secretion of apolipoprotein B-48-containing lipoproteins in subjects with type 2 diabetes. *J Lipid Res* 48:1336-1342.
- Kinoshita M, Ohnishi H, Maeda T, Yoshimura N, Takeoka Y, et al. (2009) Increased serum apolipoprotein B48 concentration in patients with metabolic syndrome. *J Atheroscler Thromb* 16:517-522.
- Kutty KM, Bryant DG, Farid NR (1978) Serum lipids in hypothyroidism--a re-evaluation. *J Clin Endocrinol Metab* 46:55-56.
- Kuusi T, Taskinen MR, Nikkilä EA (1988) Lipoproteins, lipolytic enzymes, and hormonal status in hypothyroid women at different levels of substitution. *J Clin Endocrinol Metab* 66:51-56.
- de Bruin TW, van Barlingen H, van Linde-Sibenius Trip M, van Vuurst de Vries AR, Akveld MJ, et al. (1993) Lipoprotein(a) and apolipoprotein B plasma concentrations in hypothyroid, euthyroid, and hyperthyroid subjects. *J Clin Endocrinol Metab* 76:121-126.
- Ito M, Takamatsu J, Matsuo T, Kameoka K, Kubota S, et al. (2003) Serum concentrations of remnant-like particles in hypothyroid patients before and after thyroxine replacement. *Clin Endocrinol (Oxf)* 58:621-626.
- Ito M, Takamatsu J, Sasaki I, Hiraiwa T, Fukao A, et al. (2004) Disturbed metabolism of remnant lipoproteins in patients with subclinical hypothyroidism. *Am J Med* 117:696-699.
- Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, et al. (1993) Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. *Clin Chim Acta* 223:53-71.
- Kinoshita M, Kojima M, Matsushima T, Teramoto T (2005) Determination of apolipoprotein B-48 in serum by a sandwich ELISA. *Clin Chim Acta* 351:115-120.
- Ito M, Arishima T, Kudo T, Nishihara E, Ohye H, et al. (2007) Effect of levo-thyroxine replacement on non-high-density lipoprotein cholesterol in hypothyroid patients. *J Clin Endocrinol Metab* 92:608-611.
- Mugii S, Hanada H, Takeoka K, Hidaka Y, Masuda D, et al. (2009) Clinical significance of apolipoprotein B-48 (apoB-48) in patients with thyroid disease. *Rinsho Byori* 57:1058-1063.
- Abrams JJ, Grundy SM, Ginsberg H (1981) Metabolism of plasma triglycerides in hypothyroidism and hyperthyroidism in man. *J Lipid Res* 22:307-322.
- Redgrave TG, Elsegood CL, Mamo JC, Callow MJ (1991) Effects of hypothyroidism on the metabolism of lipid emulsion models of triacylglycerol-rich lipoproteins in rats. *Biochem J* 273:375-381.
- Zerbinatti CV, Oliveira HC, Wechesler S, Quintao EC (1991) Independent regulation of chylomicron lipolysis and particle removal rates: effects of insulin and thyroid hormones on the metabolism of artificial chylomicrons. *Metabolism* 40:1122-1127.
- Weintraub M, Grosskopf I, Trostanesky Y, Charach G, Rubinstein A, et al. (1999) Thyroxine replacement therapy enhances clearance of chylomicron remnants in patients with hypothyroidism. *J Clin Endocrinol Metab* 84:2532-2536.
- Smith FR, Goodman DS (1971) The effects of diseases of the liver, thyroid, and kidneys on the transport of vitamin A in human plasma. *J Clin Invest* 50:2426-2436.
- Smith D, Watts GF, Dane-Stewart C, Mamo JC (1999) Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. *Eur J Clin Invest* 29:204-209.
- Sato I, Ishikawa Y, Ishimoto A, Katsura S, Toyokawa A, et al. (2009) Significance of measuring serum concentrations of remnant lipoproteins and apolipoprotein B-48 in fasting period. *J Atheroscler Thromb* 16:12-20.
- Ness GC, Lopez D (1995) Transcriptional regulation of rat hepatic low-density lipoprotein receptor and cholesterol 7 alpha hydroxylase by thyroid hormone. *Arch Biochem Biophys* 323:404-408.
- Staels B, Van Tol A, Chan L, Will H, Verhoeven G, et al. (1990) Alterations in thyroid status modulate apolipoprotein, hepatic triglyceride lipase, and low density lipoprotein receptor in rats. *Endocrinology* 127:1144-1152.

27. Proctor SD, Mamo JC (2003) Intimal retention of cholesterol derived from apolipoprotein B100- and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol* 23:1595-1600.
28. Pal S, Semorine K, Watts GF, Mamo J (2003) Identification of lipoproteins of intestinal origin in human atherosclerotic plaque. *Clin Chem Lab Med* 41:792-795.
29. Tanimura K, Nakajima Y, Nagao M, Ishizaki A, Kano T, et al. (2008) Association of serum apolipoprotein B48 level with the presence of carotid plaque in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 81:338-344.
30. Campos E, Nakajima K, Tanaka A, Havel RJ (1992) Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. *J Lipid Res* 33:369-380.
31. Dane-Stewart CA, Watts GF, Mamo JC, et al. (2002) Effect of Simvastatin on markers of triglyceride-rich lipoproteins in familial hypercholesterolaemia. *Eur J Clin Invest* 32:493-499.
32. Cappola AR, Ladenson PW (2003) Hypothyroidism and atherosclerosis. *J Clin Endocrinol Metab* 88:2438-2444.
33. Vanhaelst L, Neve P, Chailly P, Bastenie PA (1967) Coronary-artery disease in hypothyroidism. Observations in clinical myxoedema. *Lancet* 2:800-802.
34. Steinberg AD (1968) Myxedema and coronary artery disease--a comparative autopsy study. *Ann Intern Med* 68:338-344.
35. Bastenie PA, Vanhaelst L, Bonnyns M, Neve P, Staquet M (1971) Preclinical hypothyroidism: a risk factor for coronary heart-disease. *Lancet* 1:203-204.
36. Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, et al. (2005) Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. *Arch Intern Med* 165:2467-2472.
37. Parle JV, Maisonneuve P, Sheppard MC, Boyle P, Franklyn JA (2001) Prediction of all-cause and cardiovascular mortality in elderly people from one low serum thyrotropin result: a 10-year cohort study. *Lancet* 358:861-865.