

Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA¹

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Abstract The present study was designed to evaluate the metabolism of chylomicron and chylomicron remnants by measuring serum apolipoprotein B-48 (apoB-48) levels in 335 normolipidemic and 253 hyperlipidemic subjects using a novel ELISA system. The distribution of fasting serum apoB-48 levels in normolipidemic subjects varied widely, ranging from <1 to >24 µg/ml (mean, 5.2 ± 3.8 µg/ml; median, 3.9 µg/ml). Serum apoB-48 levels correlated with serum triglyceride (TG) concentrations ($r = 0.45$, $P < 0.001$), but not with total cholesterol levels. Serum apoB-48 levels were 7 to 18 times higher in patients with Type I, Type V, and Type III hyperlipidemia, and only slightly higher in patients with Type IIa, Type IIb, and Type IV hyperlipidemia, compared with normolipidemic subjects. The calculated apoB-48/TG ratio was elevated only in patients with dysbetalipoproteinemia (apoE2/2 phenotype). In normolipidemic subjects, oral fat loading resulted in about 2-fold increase in serum apoB-48 levels, with a peak level recorded at 3–4 h postloading, and then returned to the baseline level within 6 h. On the other hand, in patients with dysbetalipoproteinemia, serum apoB-48 levels did not change considerably. Our results indicate that serum apoB-48 is a very useful parameter for evaluating lipoprotein metabolism in exogenous pathways.—Sakai, N., Y. Uchida, K. Ohashi, T. Hibuse, Y. Saika, Y. Tomari, S. Kihara, H. Hiraoka, T. Nakamura, S. Ito, S. Yamashita, and Y. Matsuzawa. **Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA.** *J. Lipid Res.* 2003. 44: 1256–1262.

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The pathogenic role of hypertriglyceridemia in atherosclerosis has long been elusive. A recent meta analysis of several cohort studies revealed that plasma triglyceride (TG) level is an independent risk factor for cardiovascular

diseases (1). The relationship between plasma TG levels and atherosclerotic diseases has often been discussed in relation to postprandial hyperlipidemia (2–7). Several studies have pointed to the relationship between the impaired metabolism of postprandial TG-rich lipoproteins (TRLs) and the presence or development of coronary heart disease (CHD). A mechanistic hypothesis linking the postprandial generation of TRL remnants (products of lipolytic degradation of TRL produced by the liver, VLDL, and by the intestine chylomicrons) to the development of atherosclerosis was formulated almost 20 years ago by Zilversmit (8). TG-depleted remnants are considered to be atherogenic because they can penetrate the mucosal lining of the arterial wall and become entrapped within the subendothelial space. Thus, accurate evaluation of the kinetics of chylomicron and chylomicron remnants is very important.

Chylomicrons are secreted by the intestine after fat ingestion. Chylomicron particles contain apolipoprotein B-48 (apoB-48) as the structural protein, which in humans is formed exclusively in the intestine after tissue-specific editing of the apoB-100 mRNA (9, 10). Different methods are used for estimation of postprandial lipoproteins. A number of studies have made use of retinyl palmitate (RP) labeling of chylomicrons, together with postprandial plasma TG quantification. The RP technique has shortcomings as it has recently been shown that RP can exchange between lipoprotein species in plasma, implying

Abbreviations: CHD, coronary heart disease; CV, coefficient of variation; LPL, lipoprotein lipase; RLP, remnant-like particle; RP, retinyl palmitate; TC, total cholesterol; TG, triglyceride; TRL, TG-rich lipoprotein.

¹ The anti-human apolipoprotein B-48 monoclonal antibodies (B-48-151) are available upon a written request to N.S. for research use only and will be commercially available in the future.

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that plasma RP is not always synonymous with the presence of chylomicron remnants (11). Furthermore, the RP does not provide uniform labeling of the chylomicrons, as it is probable that the larger species carry more RP than do the smaller ones. In contrast, the use of apoB-48 and apoB-100 quantification in TRL provides a good estimate of lipoprotein particle numbers. Therefore, a more direct assay of apoB-48 level has been through the monitoring of the kinetics of chylomicrons and their remnants *in vivo*; however, their quantification has been limited because of their low concentrations in plasma and lack of specific antibodies.

A wide range of fasting apoB-48 values has been reported by different groups (11–17), likely because no standard method has yet been established. Furthermore, the results are expressed as either TRL apoB-48 or plasma apoB-48. These studies used the following assays for estimation of apoB-48 levels: SDS-PAGE (11–13), SDS-PAGE coupled with Western blotting (15, 16), and a competitive ELISA with polyclonal antibodies (14, 17). The Western blotting method, however, is very time-consuming and is less quantitative than the ELISA method, and the continuous supply of polyclonal antibodies of equal reactivity for long-term surveys remains questionable. In this regard, we recently developed monoclonal antibodies against apoB-48 and reported their specificity and possible usage for ELISA assay system (18).

In the present study, we report a novel ELISA system for measurement of serum apoB-48. We also used the novel assay to measure fasting and postprandial serum apoB-48 levels in 335 normolipidemic and 253 subjects with various forms of hyperlipidemia. We confirm that the fasting apoB-48 concentration determined by our ELISA system closely represents lipoprotein metabolism in exogenous pathways.

MATERIALS AND METHODS

Subjects

The subjects investigated were a consecutive series of 574 healthy volunteers who were employees of a single company and visited the hospital for annual general health checkup. Of the total sample, 335 subjects were considered normolipidemic [total cholesterol (TC) < 220 mg/dl and TG < 150 mg/dl], while the remaining 239 healthy subjects were considered hyperlipidemic (TC \geq 220 mg/dl and/or TG \geq 150 mg/dl). The phenotype of hyperlipidemic subjects was also determined based on the criteria proposed by the Research Committee for Primary Hyperlipidemia of the Ministry of Health and Welfare of Japan: 1) Type IIa hyperlipidemia: in the presence of hypercholesterolemia (TC \geq 220 mg/dl and TG < 150 mg/dl); 2) Type IIb hyperlipidemia: in the presence of both hypercholesterolemia and hypertriglyceridemia (TC \geq 220 mg/dl and TG \geq 150 mg/dl), but serum TG level was < 500 mg/dl; 3) Type IV hyperlipidemia: in the presence of hypertriglyceridemia (TC < 220 mg/dl and TG \geq 150 mg/dl), but serum TG level was < 500 mg/dl; and 4) Type V hyperlipidemia: in the presence of both hypercholesterolemia and hypertriglyceridemia (TC \geq 220 mg/dl and TG \geq 150 mg/dl), but serum TG level was > 500 mg/dl. Two patients with lipoprotein lipase (LPL) deficiency (Type I hyperlipidemia), five pa-

tients with familial dysbetalipoproteinemia (Type III hyperlipidemia), and seven Type V hyperlipidemic patients who were referred to Osaka University Hospital for the examination of hyperlipidemia were also added to the hyperlipidemic group, because the original hyperlipidemic group contained no Type I, no Type III, and only one subject with Type V hyperlipidemia. LPL deficiency was determined by measuring LPL activity in postheparin plasma 15 min after injection of sodium heparin (50 IU/kg body weight). Familial dysbetalipoproteinemia was diagnosed based on the presence of hypercholesterolemia and hypertriglyceridemia, an apoE2/2 phenotype, and the presence of a broad β pattern on polyacrylamide gel electrophoresis of serum lipoproteins. The clinical and lipid profiles of 253 hyperlipidemic and 335 normolipidemic subjects are shown in **Table 1**. Ten out of 335 normolipidemic subjects and four (Cases 1–4) out of five familial dysbetalipoproteinemic subjects agreed to enroll in another study for evaluation by oral fat loading. All subjects gave their informed consent to participate in this study.

Lipid and lipoprotein analyses

Blood was collected in a glass tube after at least a 12 h overnight fast. Serum was separated by low-speed centrifugation (15 min, 2,000 *g* at 4°C), aliquoted, and frozen at –80°C until used for determination of apoB-48. The rest of the serum sample was kept at 4°C for further analyses that were carried out within 1 week. Serum TC and TG levels were determined by enzymatic methods. Serum HDL cholesterol level was determined by the homogeneous method (19). The presence of a broad β pattern of serum lipoproteins was determined by PAGE (JOKOH Co., Tokyo, Japan). ApoE phenotype was determined by isoelectric focusing gel electrophoresis (JOKOH Co.).

Measurement of serum apoB-48 levels by ELISA

Serum apoB-48 levels were measured by a sandwich ELISA using anti-human apoB-48 monoclonal antibodies (designated B-48-151) as reported previously with minor modification (18). A 96-well microtiter plate (NUNC A/S, Roskilde, Denmark) was coated with anti-apoB-48 monoclonal antibodies by incubating overnight at 4°C, followed by washing with PBS and blocking with PBS containing 5% (w/v) BSA. Serum samples were diluted 1:20 with 1% (w/v) BSA-0.1% (w/v) Tween 20-PBS, and 100 μ l of the diluted serum was used for the assay. The alkaline phosphatase-conjugated anti-human apoB monoclonal antibodies (designated B-100-228) were used as the second antibody. The culture media of rat hepatoma McA-RH7777 cell lines stably transfected with human apoB-48 cDNA (kindly provided by Dr. Zemin Yao) (20, 21) were aliquoted, frozen, and used as a working standard because they could be prepared in large amounts easily and constantly. In each assay, the working standard was run with each set of serum samples, and a standard curve was constructed. The linear range and correlation coefficient of the standard curve were up to 28 μ g and >0.99, respectively. The serum samples were diluted if needed so as to be measured in the linear range. The specificity of the assay was described previously (18). The intra-assay coefficients of variation (CVs) for sera with high, intermediate, and low concentrations were 7.1%, 5.5%, and 9.1%, respectively (*n* = 12). The interassay CVs were 9.8%, 10.0%, and 10.7%, respectively (*n* = 6).

The apoB-48 concentration of the working standard was determined using purified human apoB-48 as the primary standard. The apoB-48 was purified from human plasma by affinity chromatography. Briefly, the *d* < 1.006 fraction was separated by ultracentrifugation according to the method of Havel et al. (22). The *d* < 1.006 fraction was passed through the anti-apoB-100 (designated 80C-3613) affinity column three times to exclude apoB-100-containing TRL. The apoB-48 was purified from the

TABLE 1. Characteristics of hyperlipidemic and normolipidemic subjects investigated

	n	Sex	Age	TC	TG	HDL Cholesterol	ApoB-48	ApoB-48:TC Ratio
					mg/dl		µg/ml	
Type I	2	M	37	506	2,506	42	92.4	0.037
		M	55	171	1,210	23	34.3	0.028
Type IIa								
Male	78		49.0 ± 5.3	246 ± 24 ^g	105 ± 23 ^g	58 ± 16 ⁱⁱ	6.6 ± 3.9 ^h	0.064 ± 0.036
Female	20		51.2 ± 5.4 ^k	247 ± 19 ^j	100 ± 33 ^k	69 ± 12	5.8 ± 3.5 ^l	0.060 ± 0.034
Total	98		49.5 ± 5.3 ^c	247 ± 23 ^a	104 ± 26 ^a	60 ± 16 ^c	6.4 ± 3.8 ^b	0.063 ± 0.035
Type IIb	54	M = 52, F = 2	48.7 ± 4.6	242 ± 22 ^g	213 ± 61 ^g	49 ± 10 ⁱ	11.6 ± 6.2 ^g	0.055 ± 0.028
Type III								
Case 1		M	74	386	452	73	68.3	0.151
Case 2		M	65	296	342	46	48.0	0.140
Case 3		M	57	248	374	48	59.5	0.159
Case 4		F	50	354	420	50	54.2	0.129
Case 5		F	62	305	303	58	51.9	0.172
Total	5		59.0 ± 7.9 ^c	335 ± 42 ^b	379 ± 69 ^b	57 ± 12	56.4 ± 7.9 ^b	0.150 ± 0.016 ^b
Type IV	86	M = 86	49.2 ± 5.7	193 ± 20 ^h	223 ± 76 ^g	46 ± 12 ^g	12.7 ± 9.4 ^g	0.056 ± 0.033
Type V	8	M = 6, F = 2	44.9 ± 8.3	302 ± 56 ^a	906 ± 99 ^a	44 ± 12	37.7 ± 14.9 ^a	0.041 ± 0.018
Control								
Male	281		47.6 ± 7.1 ^f	185 ± 23	90 ± 28 ^d	53 ± 12 ^d	5.4 ± 3.9 ^e	0.060 ± 0.036
Female	54		45.9 ± 4.5	183 ± 20	67 ± 30	66 ± 14	3.7 ± 2.8	0.059 ± 0.033
Total	335		47.3 ± 6.8	185 ± 23	87 ± 29	55 ± 14	5.2 ± 3.8	0.060 ± 0.035

F, female; M, male; TC, total cholesterol; TG, triglyceride. Mean ± SD.

^a $P < 0.0001$ versus control.

^b $P < 0.001$ versus control.

^c $P < 0.01$ versus control.

^d $P < 0.0001$ male versus female.

^e $P < 0.001$ male versus female.

^f $P < 0.01$ male versus female.

^g $P < 0.0001$ versus male control.

^h $P < 0.01$ versus male control.

ⁱ $P < 0.05$ versus male control.

^j $P < 0.0001$ versus female control.

^k $P < 0.001$ versus female control.

^l $P < 0.05$ versus female control.

unbound fraction using the anti-apoB-48 (B-48-151) affinity column. The purity of the purified apoB-48 was confirmed to be ~97% on SDS-PAGE with Coomassie staining. The apoB-48 concentration of the primary standard was determined with BCA protein assay reagent (Pierce Biotechnology, Inc., Rockford, IL), and by the densitometric comparison of the band on the SDS-PAGE gel with a known concentration of human apoB-100 (LDL of the density range from 1.030 to 1.050), provided that the chromogenicities of apoB-48 and apoB-100 were considered the same as reported previously (23). The apoB-48-rich TRL, the unbound fraction of the anti-apoB-100 affinity column chromatography of the $d < 1.006$ fraction as described above, was also used to determine the concentration of the working standard, and yielded the same results as the purified apoB-48.

Oral fat loading test

OFT cream (JOMO Food Co., Gunma, Japan) containing 35% fat without sugar was given after a 12 h overnight fast. Each study subject took 30 g fat/m² body surface area within 3 min. The study subjects were allowed to consume only a small amount of water during the study period to protect against dehydration. Blood was drawn before and 1 h, 2 h, 3 h, 4 h, and 6 h after the fat load, followed by immediate serum separation.

Statistical analyses

All data were expressed as mean ± SD. Statistical analyses were performed using StatView statistical software (Hulinks Inc., Tokyo, Japan). The Mann-Whitney U test, a distribution-free nonparametric test, was used to compare the statistical differences between groups. Spearman correlation coefficients (r)

were determined to assess the relationship between different parameters. Statistical significance was established at $P < 0.05$.

RESULTS

Figure 1 shows the distribution of fasting serum apoB-48 levels in 335 normolipidemic subjects. The apoB-48 levels were distributed over a wide range from < 1 µg/ml (0.1 mg/dl) to > 24 µg/ml (2.4 mg/dl). The mean level in this group was 5.2 ± 3.8 µg/ml, the median was 3.9 µg/ml, and the 95th percentile range for all subjects was ≤ 13 µg/ml.

To investigate the correlation between serum apoB-48 levels and TC and TG concentrations, serum apoB-48 levels were plotted against serum TC (**Fig. 2B**) and TG (**Fig. 2A**) concentrations in 335 normolipidemic subjects. Serum apoB-48 levels correlated positively with serum TG levels ($r = 0.45$, $P < 0.001$), but there was no significant correlation between serum apoB-48 and TC levels.

In order to investigate the significance of measuring serum apoB-48 levels in the evaluation of lipoprotein metabolism, the fasting serum apoB-48 levels in patients with various phenotypes of hyperlipidemia were determined (**Fig. 3A**). Relative to the control, apoB-48 levels were seven to 18 times higher in the two subjects with Type I (34.3 and 92.4 µg/ml) and the eight patients with Type V

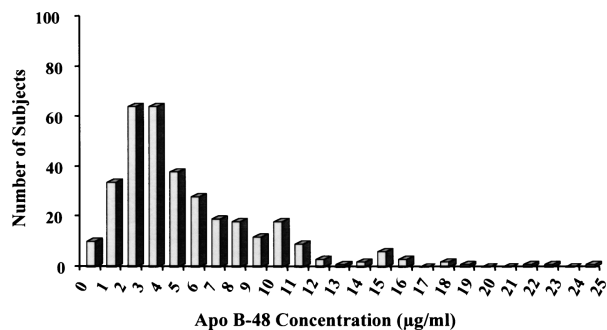


Fig. 1. Distribution of fasting serum apolipoprotein B-48 (apoB-48) levels in normolipidemic subjects. Serum apoB-48 levels were determined in 335 normolipidemic subjects. The mean \pm SD value was 5.2 ± 3.8 $\mu\text{g/ml}$ and the median was 3.9 $\mu\text{g/ml}$.

(37.7 ± 14.9 $\mu\text{g/ml}$) hyperlipidemia, consistent with increased chylomicrons. Subjects with familial dysbetalipoproteinemia also had high levels of serum apoB-48 (56.4 ± 7.9 $\mu\text{g/ml}$, $n = 5$), suggesting that chylomicron remnants accumulated in dysbetalipoproteinemic serum. The apoB-48 levels in Type IIa (6.4 ± 3.8 $\mu\text{g/ml}$, $n = 98$), Type IIb (11.6 ± 6.2 $\mu\text{g/ml}$, $n = 54$), and Type IV (12.7 ± 9.4 $\mu\text{g/ml}$, $n = 86$) hyperlipidemic subjects were significantly higher than the control, but such increase was small in comparison with the above three groups.

The ratio of serum apoB-48 levels to serum TG concentrations (apoB-48/TG ratio) was calculated in subjects with various forms of hyperlipidemia and in normolipidemic subjects, and is shown in Fig. 3B. Although the apoB-48/TG ratio was within the normal range in Type I and Type V hyperlipidemic subjects, it was high in patients with dysbetalipoproteinemia (0.150 ± 0.016 , $P < 0.001$ vs. Control). The apoB-48/TG ratio was more than 0.12 in all five subjects.

In order to investigate whether the measured fasting apoB-48 levels represent lipoprotein metabolism in exogenous pathway, the oral fat loading test was carried out in

10 normolipidemic and four familial dysbetalipoproteinemic subjects. **Figure 4** shows the serum TG level (Fig. 4A), apoB-48 level (Fig. 4B), and apoB-48/TG ratio (Fig. 4C) in normolipidemic or dysbetalipoproteinemic subjects at the indicated points. In normolipidemic subjects, serum apoB-48 levels increased to about 2-fold, with the peak measured at 3–4 h after oral fat loading, then returned to the baseline level within 6 h. In the same group, the mean fasting levels of apoB-48 were 5.7 ± 2.8 $\mu\text{g/ml}$, and the mean postprandial peak levels were 10.4 ± 3.0 $\mu\text{g/ml}$. The serum apoB-48 levels ran parallel to postprandial TG response, leading to a stable apoB-48/TG ratio. The fasting and postprandial serum TG levels in subjects with familial dysbetalipoproteinemia were significantly higher at any time point with a prolonged elevation relative to those of normolipidemic subjects. The postprandial apoB-48 levels in dysbetalipoproteinemic subjects remained at high levels and appeared to be relatively stable, and thus, the apoB-48/TG ratios steadily decreased after oral fat loading.

DISCUSSION

Several investigators have reported a wide range of fasting apoB-48 levels from 0.08 $\mu\text{g/ml}$ to 60 $\mu\text{g/ml}$ (11–17), within which our value (5.2 ± 3.8 $\mu\text{g/ml}$) also falls. The wide range probably stems from the lack of a standardized method of measurement and the expression of the results as either TRL apoB-48 or plasma apoB-48. Among the available methods, the ELISA system with monoclonal antibodies described here is expected to yield reproducible values without the time-consuming effort of ultracentrifugation. In the normolipidemic subjects with high fasting serum apoB-48 levels, we could not perform repeated measurements of apoB-48 on the same subjects because the samples were anonymous. Since certain conditions, especially alcohol consumption and consumption of carbo-

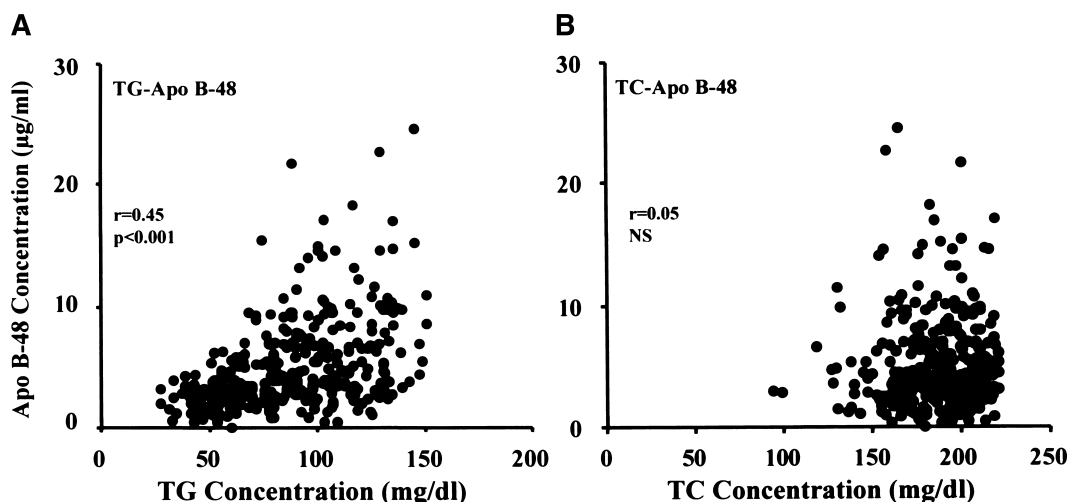


Fig. 2. Correlation between serum apoB-48 levels and total cholesterol (TC) or triglyceride (TG) levels. Fasting serum apoB-48 levels were plotted against serum TGs (A) and TC (B) concentrations. The correlations between serum apoB-48 and serum TG or TC were assessed by Spearman's correlation coefficient.

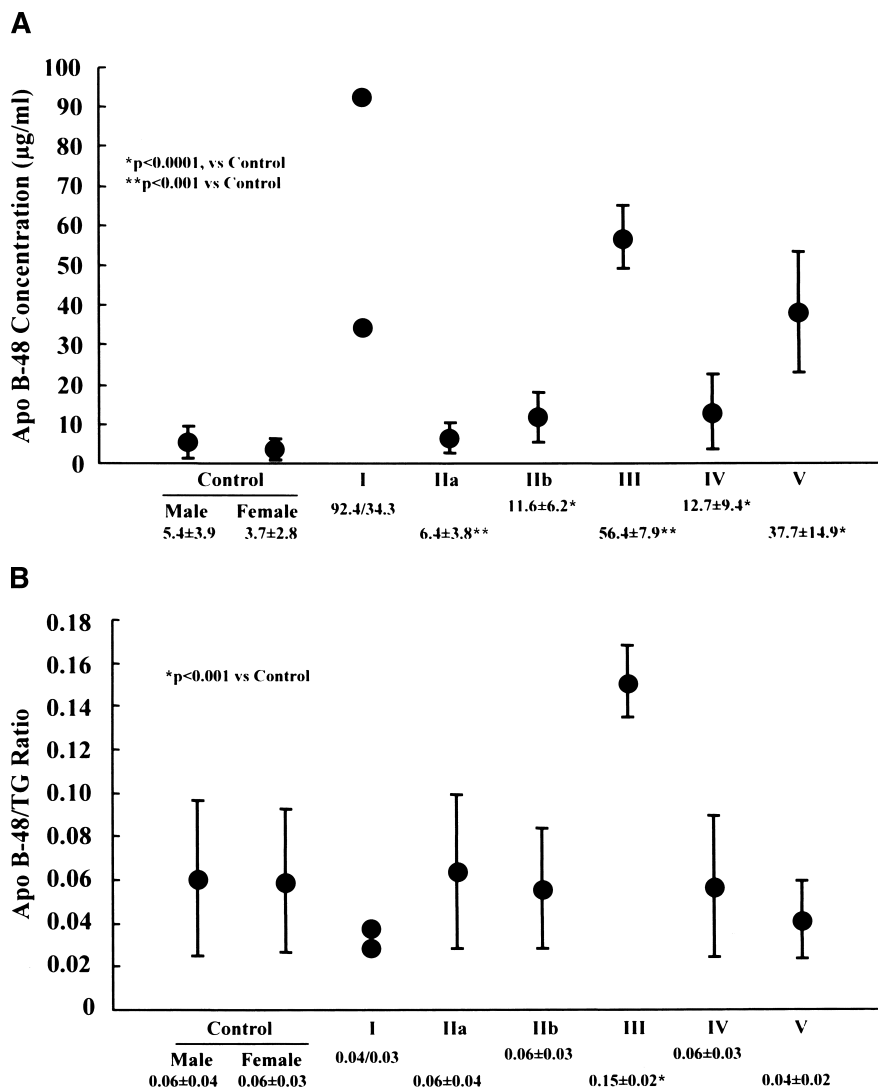


Fig. 3. Fasting serum apoB-48 levels in various types of hyperlipidemia. Fasting apoB-48 levels (A) and apoB-48/TG ratio (B) were determined in 335 normolipidemic subjects (281 males and 54 females), and Type I (n = 2), Type IIa (n = 98), Type IIb (n = 8), Type III (n = 5), Type IV (n = 86), and Type V (n = 8) hyperlipidemic subjects. Data are expressed as mean \pm SD.

hydrates and failure to strictly follow a 12 h fasting, may influence the fasting levels of apoB-48, further studies are needed to assess the significance of increased levels of fasting serum apoB-48 in these subjects. We speculate that the high fasting serum apoB-48 concentrations reflect the impaired removal of chylomicron and/or chylomicron remnants from the circulation (postprandial hyperlipidemia), and this speculation is supported by the previous findings of Smith et al. (16), demonstrating a strong relationship between fasting plasma concentrations of apoB-48 and postprandial kinetics of apoB-48, retinyl ester, and TG. Since the relation of postprandial hyperlipidemia and the presence or development of CHD is well established, it will be also intriguing to investigate whether fasting serum apoB-48 level is also related to the occurrence of CHD.

The high serum apoB-48 levels in patients with dysbetalipoproteinemia who do not have high levels of chylomicrons clearly reflect accumulation of atherogenic chylomicron

remnants. The apoB-48/TG ratio enabled the discrimination of dysbetalipoproteinemia from Type I and Type V hyperlipidemia. Since one chylomicron particle contains only one molecule of apoB-48, serum apoB-48 concentrations represent the number of chylomicrons and chylomicron remnant particles. The high serum apoB-48 levels and high apoB-48/TG ratio observed in patients with dysbetalipoproteinemia indicate an increased number of smaller particles, while the high apoB-48 levels and low apoB-48/TG ratio observed in Type I and Type V hyperlipidemia reflect both an increased number of the apoB-48-containing particles and enrichment of the particles with TG. These results are consistent with elevated levels of chylomicrons in Type I and Type V hyperlipidemia and of chylomicron remnants in dysbetalipoproteinemia.

It is sometimes difficult to distinguish dysbetalipoproteinemia from Type IIb or Type V hyperlipidemia based

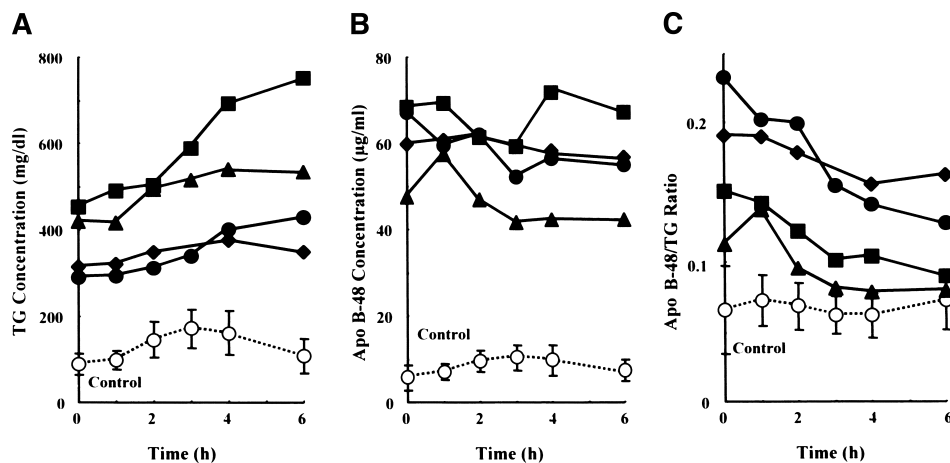


Fig. 4. Fasting and postprandial serum TG and apoB-48 levels and apoB-48/TG ratio after oral fat loading. Oral fat loading test was performed in 10 normolipidemic and four familial dysbetalipoproteinemic subjects as described in Materials and Methods. Serum TG (A) and apoB-48 (B) levels and apoB-48/TG ratios (C) were determined before and 1 h, 2 h, 3 h, 4 h, and 6 h after the fat loading. Open circles and dotted lines represent the responses of normolipidemic subjects. Data are expressed as mean \pm SD. Closed symbols represent the four patients with familial dysbetalipoproteinemia who agreed to participate in this study.


on serum lipid levels only. Since familial dysbetalipoproteinemia is a very atherogenic condition when untreated, patients with this condition should be more strictly controlled with respect to lifestyle and other worsening factors to protect them against or to delay the development of atherosclerotic diseases. For this purpose, accurate diagnosis of this form of hyperlipidemia is strongly required. A couple of convenient methods for the diagnosis of dysbetalipoproteinemia have been proposed to spare the time-consuming electrophoresis procedure and phenotyping of apoE. These include a VLDL cholesterol-to-serum total TG ratio of ≥ 0.30 and remnant-like particle (RLP) cholesterol-to-serum total TG ratio of ≥ 0.10 , both of which reflect the enrichment of TRL in cholesterol, a characteristic feature of β -VLDL, and the latter is reported to be more sensitive (24). RLP cholesterol is thought to represent a mixture of cholesterol in apoB-48-containing lipoproteins and of a certain subpopulation of VLDL (apoB-100-containing lipoprotein) enriched with apoE and cholesteryl ester. Thus, serum apoB-48 levels and the apoB-48/TG ratio in combination with serum lipid concentrations could also provide an accurate and simple phenotypic diagnosis of dysbetalipoproteinemia.

Subjects with Type IIa, Type IIb, and Type IV hyperlipidemia showed only a slight increase in serum apoB-48 levels, despite elevated TG levels; however, it is noted that several subjects with phenotypic Type IV hyperlipidemia had almost the same levels of apoB-48 as those with Type V hyperlipidemia (data not shown). The results indicate impairment of lipid metabolism in the exogenous pathway and accumulation of chylomicrons and/or chylomicron remnants in these subjects. In this respect, Type IV hyperlipidemic subjects with elevated apoB-48 levels should be classified as Type V hyperlipidemic even though they have normal serum TC levels. Thus, this overlap probably indicates the limitation of the phenotypic classification

of hyperlipoproteinemia based on serum TC and TG levels, especially with regard to patients with hypertriglyceridemia.

The pattern of apoB-48 response after oral fat loading paralleled that of TG response in normolipidemic subjects, suggesting that serum apoB-48 concentrations closely represent exogenous lipoprotein metabolism in the postprandial state. The peak serum apoB-48 levels (2-fold increase) and the mean latency-to-peak level of 3–4 h after fat loading are almost consistent, in keeping with those reported previously by other investigators (11–17, 25). On the other hand, serum apoB-48 levels in dysbetalipoproteinemic subjects did not parallel serum TG concentrations that showed a prolonged increase and delayed latency in the postprandial state. The disproportionate change of TG and apoB-48 levels in dysbetalipoproteinemia was confirmed by a continuous decrease in the apoB-48/TG ratio after the fat loading. Compared with the response of apoB-48 in normolipidemic subjects, the relatively stable levels of serum apoB-48 after fat loading in dysbetalipoproteinemia is of interest. The lack of increase in apoB-48 levels after fat loading may reflect only the masking effect of a slight increase by the existing very high fasting levels. Nevertheless, the continuous decline of the apoB-48/TG ratio during the test indicates that the high TG levels after fat loading in dysbetalipoproteinemia are more predominantly attributed to enrichment of lipoprotein particles with TG than to increased circulating particle numbers. The mechanism(s) of the discrepancy between subjects with normolipidemia and dysbetalipoproteinemia in response of apoB-48 and TG after fat loading needs further investigation.

In conclusion, in the present study we established a novel ELISA system for measurement of serum apoB-48 and demonstrated the significance of such measurement for the evaluation of lipoprotein metabolism in exoge-

nous pathways. Serum apoB-48 level may become a simple tool for the assessment of postprandial hyperlipidemia, one of the major risk factors for coronary atherosclerosis. Further studies are needed to investigate the relationship between serum apoB-48 levels and atherosclerotic cardiovascular diseases. 

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