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ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 1536–1542
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Fasting and postprandial apolipoprotein B-48 levels in healthy, obese, and hyperlipidemic subjects

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Received 1 December 2008; accepted 2 April 2009

Abstract

Apolipoprotein (apo) B-48 is the only specific marker of intestinal lipoproteins. We evaluated a novel enzyme-linked immunosorbent assay (ELISA) standardized with recombinant apo B-48 to measure apo B-48 in plasma and triglyceride-rich lipoproteins (TRLs, density <1.006 g/mL). Coefficients of variation were less than 2.5%. Assay values correlated well ($r = 0.82$, $P < .001$) with values obtained by gel scanning of TRLs (n = 75 samples); however, the gel scanning method yielded values that were about 50% lower than ELISA values. About 60% to 70% of apo B-48 was found in TRLs. In 12 healthy subjects, median fasting plasma apo B-48 levels were 0.51 mg/dL and were increased by 121% to 147% in the fed state. In 63 obese subjects, median fasting apo B-48 values were 0.82 mg/dL; and feeding resulted in almost no change in total cholesterol, non–high-density lipoprotein cholesterol, or total apo B values, whereas triglyceride, remnant lipoprotein cholesterol, and apo B-48 levels were significantly higher ($P < .05$; by +73%, +58%, and +106%), and direct low-density lipoprotein cholesterol and direct high-density lipoprotein cholesterol were significantly lower ($P < .001$, by −13% and −20%) than fasting values. Relative to controls, 270 hyperlipidemic subjects had significantly higher ($P < .001$, +115%) fasting total apo B and higher apo B-48 values ($P = .06$, +37%). Our data indicate that the apo B-48 ELISA tested provides highly reproducible results and is excellent for research studies. Median apo B-48 values in healthy subjects are about 0.5 mg/dL and increase more than 100% in the fed state. Elevated levels are observed in obese and hyperlipidemic subjects.
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1. Introduction

Our major purpose in this investigation was to evaluate a novel immunoassay for apolipoprotein (apo) B-48. Apolipoprotein B-48 is the only known specific marker of intestinal lipoproteins [1-6]. Retinyl palmitate has been suggested as a marker of intestinal lipoproteins; but this constituent can exchange between lipoprotein particles, in contrast to the B apolipoproteins [7-12]. Apolipoprotein B-48's molecular weight is 264 000 Da (or 48% of that of apo B-100, with a known molecular weight of 550 000 Da). Apolipoprotein B-48 is the constitutive protein of chylomicrons. It cannot be secreted by the intestine without first being bound to triglyceride (TG) via the action of microsomal transfer protein. If microsomal transfer protein is not normally synthesized, then no detectable apo B-48 or apo B-100 is found in plasma, as in abetalipoproteinemia. Our own data using stable isotope metabolic studies indicate that about 2 mg/kg/d of apo B-48 is secreted into the plasma space in humans, with a mean plasma residence time of approximately 5 hours [1]. Newly formed chylomicrons are very rich in TG and contain apo B-48, apo A-I, apo A-IV, and the C apolipoproteins. Chylomicrons and very low-density lipoproteins lose much of their TG in the plasma space through the action of lipoprotein lipase. During this process, the C apolipoproteins including apo C-II, which activates lipoprotein lipase, and apo C-III, which inhibits this enzyme, as well as apo A-I and apo A-IV are transferred to high-density lipoproteins (HDLs). In addition, some TGs are transferred to HDLs in exchange for cholesteryl ester via the action of cholesteryl ester transfer protein. If lipoprotein lipase is defective, then there is a lack of chylomicron

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0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.04.040

clearance, marked hypertriglyceridemia, and an increased risk of developing pancreatitis [1].

Apolipoprotein B-48 lacks the low-density lipoprotein (LDL) receptor binding domain in contrast to apo B-100, but chylomicron remnants containing apo B-48 pick up apo E from HDLs during lipolysis. Through the process of lipolysis and because of the action of cholesteryl ester transfer protein, these particles become cholesteryl ester–rich chylomicron remnants containing both apo B-48 and apo E. It has been documented that human chylomicron apo B-48 is not converted to LDLs, but is directly catabolized from the plasma space, mainly by the liver. The presence of apo E on chylomicron remnants allows for their rapid clearance by the liver via the LDL receptor, as well as other receptors involved in triglyceride-rich lipoprotein (TRL) catabolism. In the absence of apo E, there is a marked delay in the fractional catabolism of apo B-48 from TRL [2]. It has been reported that remnant lipoproteins of both hepatic and liver origin are potentially atherogenic and that apo B-48 is retained in the arterial wall along with apo B-100 [3].

Since the description of apo B-48 by Kane and colleagues [4], this protein has generally been assessed by isolating lipoproteins of density less than 1.006 g/mL or TRLs in both the fasting and fed states. Triglyceride-rich lipoprotein total apo B is then measured by immunoassay; and the relative amount of apo B-48 is assessed by carrying out gel electrophoresis on the TRL fraction, followed by gel scanning, to estimate the relative apo B-48 content [4-6]. The difficulty with this methodology is that it assumes similar chromogenicity between apo B-100 and apo B-48, which is probably not the case. Moreover, this type of assessment does not allow for an accurate measurement of plasma or serum apo B-48 concentrations. For this reason, scientists have developed monoclonal antibodies specific for apo B-48 to establish immunoassays for the direct measurement of apo B-48 in plasma and TRL [13-15]. Our purpose in this investigation was to (1) evaluate a commercially available research assay for apo B-48, (2) compare the results with values obtained by gel scanning of TRL, and (3) apply this assay to a large number of human subjects with varying lipid levels in both the fasting and fed states.

2. Methods

2.1. Study subjects

We measured changes in biochemical parameters in plasma after an overnight 12-hour fast in 354 subjects and after a variety of fat-rich meals in a number of different studies in 84 subjects. Subjects were not taking lipid-lowering medications; and those with heart disease, liver disease, kidney disease, thyroid disease, and diabetes were excluded. All subjects provided informed consent, and the research protocols were approved by the Human Investigation Review Committee of Tufts University and Tufts Medical Center. No subjects experienced any significant adverse event that was related to these studies.

In the first study (Table 1 and Fig. 1), 9 subjects (5 men and 4 postmenopausal women; mean age, 55 years; mean body mass index [BMI], 28.07 kg/m^2) with combined hyperlipidemia (LDL or LDL cholesterol [LDL-C] >130 mg/dL and TG >150 mg/dL) were sampled in the fasting state and at 3 and 6 hours during constant hourly feeding (1/20th of daily caloric intake on an average American diet) as part of a stable isotope kinetic study as previously described [8]. In this study, we also isolated TRL of density less than 1.006 g/mL by ultracentrifugation and measured total B by enzyme-linked immunosorbent assay (ELISA) and apo B-48 by gel electrophoresis and gel scanning as previously described [16].

In the second study (Table 2 and Fig. 2), we assessed changes in various lipid parameters in the fasting state as well as at 4 hours after a standard breakfast and at 8 hours after breakfast and 4 hours after a standard lunch in 12 healthy subjects on an isocaloric average American diet. All these subjects were men, with a mean age of 35.8 years and a mean BMI of 24.5 kg/m^2.

In the third study (Table 3 and Fig. 3), we measured changes in biochemical parameters in the fasting state and in the fed state in the late afternoon when subjects had already consumed breakfast (8 hours previously) and lunch (4 hours previously) while on an isocaloric average American diet (15% protein, 50% carbohydrate, and 35% fat, containing 15% of calories as saturated fat, 15% of calories as monounsaturated fat, and 5% of calories as polyunsaturated fat, with 180 mg of cholesterol per 1000 calories). These subjects were selected to be between the ages of 25 and 65 years, and all women were required to be postmenopausal. Moreover, subjects were selected to have a BMI greater than 28.0 kg/m^2. Sixty-three overweight and obese subjects

Table 1
Mean values in plasma, TRL, and TRL/plasma ratios of variables at 0, 3, and 6 hours during small frequent feeding in combined hyperlipidemic subjects

Variable / Time point/ sample/ratio	TC, mg/dL	TG, mg/dL	Apo B, mg/dL	Apo B-48, mg/dL
0 h				
Plasma	241.0 (23.1)	280.8 (65.6)	112.2 (13.2)	1.79 (0.47)
TRL	35.8 (9.6)	196.1 (44.5)	9.7 (2.5)	1.10 (0.29)
TRL/plasma ratio	0.15 (0.04)	0.70 (0.05)	0.10 (0.19)	0.63 (0.11)
3 h				
Plasma	233.4 (26.2)	272.8 (80.0)	108.9 (10.0)	2.01 (0.74)
TRL	38.2 (14.0)	208.1 (70.5)	10.1 (2.5)	1.25 (0.59)
TRL/plasma ratio	0.17 (0.07)	0.76 (0.05)	0.10(0.25)	0.65 (0.18)
6 h				
Plasma	230.6 (24.8)	277.1 (52.7)	113.1 (13.4)	2.04 (0.94)
TRL	39.1 (11.5)	213.7 (50.7)	10.3 (2.9)	1.38 (0.54)
TRL/plasma ratio	0.17 (0.07)	0.77 (0.06)	0.10 (0.21)	0.71 (0.21)

Data presented on 9 subject are means (SD). Median fed TRL apo B-48 as assessed in gel electrophoresis was 0.65 mg/dL vs a mean of 1.35 mg/dL for apo B-48 ELISA.

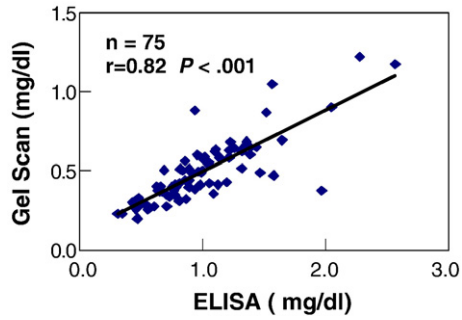

Fig. 1. Correlations between ELISA and gel scan with apo B-48 levels in TRL samples are shown.

were studied (30 men and 33 women), with a mean age of 55.5 years and a mean BMI of 33.2 kg/m^2.

In the fourth study (Table 4), 270 subjects selected for hyperlipidemia (LDL-C >160 mg/dL) were assessed in the fasting state only. These subjects included 137 men and 133 women, with a mean age of 57.0 years and a mean BMI of 29.5 kg/m^2.

2.2. Biochemical measurements

The ELISA for apo B-48 was obtained from Shibayagi (Gunma, Japan; www.shibayagi.co.jp). The characteristics and development of the apo B-48 assay have been previously described [14,15]. The assay uses monoclonal antibody raised against apo B-48 C terminal decapeptide and has been calibrated using recombinant apo B-48 antigen [15]. The investigators report no cross-reactivity with apo B-100 as verified by ELISA and Western blotting, with more than 90% recovery of apo B-48 and assay within- and between-run coefficients of variation of 4.8% and 5.4%, respectively. The assay was tested in 18 healthy volunteers in the fasting state with mean reported values of 0.460 mg/dL, with a standard deviation of 0.154 mg/dL. In 5 healthy volunteers tested in the fasting state and at 1, 2, 3, 4, 5, 6, and 8 hours after a 40-g/kg fat meal, serum apo B-48 increased about

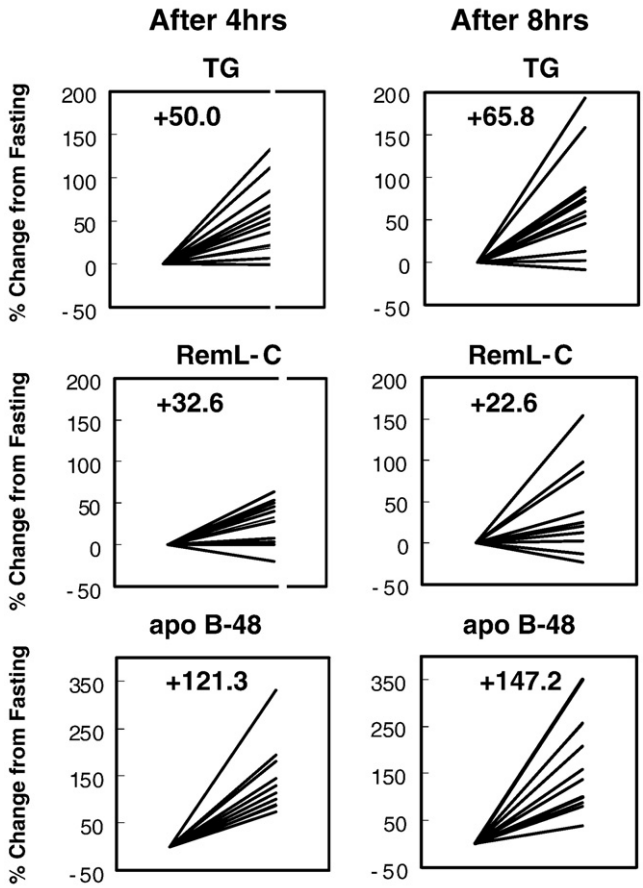

Fig. 2. Variability in postprandial responses of TG, RemL-C, and apo B-48 levels at 4 and 8 hours with healthy subjects are shown.

100%, with values peaking at 3 to 4 hours, similar to serum TG values [15].

In our hands, this assay had a sensitivity of 0.25 ng/mL; and we could not detect any cross-reactivity with human apo B-100. We documented a linear dynamic range at 2.5 to 40 ng/mL. Interferences with assay values were noted at

Table 2
Mean values at fasting and the percentage change and *P* value postprandial in healthy subjects

Variable	Fasting	Postprandial			
		4 h	% Change	8 h	% Change
TC, mg/dL	184.9 (21.4)	181.7 (22.6)	−1.8 (3.3)*	182.2 (21.9)	−1.4 (5.3)*
TG, mg/dL	90 [71-117]	116 [97-159]	+50 [21-76]†	132 [90-230]	+65.8 [30-86]†
HDL-C, mg/dL	52.4 (8.6)	49.2 (9.3)	−6.3 (6.6)†	47.7 (10.5)	−9.6 (8.8)†
TC/HDL-C ratio	3.6 (0.8)	3.8 (1.0)	+5.3 (9.2)†	4.1 (1.3)	+10.1 (14.1)†
Non–HDL-C, mg/dL	132.5 (23.9)	132.5 (25.9)	−0.2 (4.8)*	134.5 (27.6)	+1.3 (7.5)*
LDL-C, mg/dL	111.8 (20.8)	105.6 (21.2)	−5.6 (4.2)†	105.2 (20.5)	−6.0 (5.5)†
RemL-C, mg/dL	5.4 [3-7]	5.9 [4-8]	+32.6 [6-48]†	5.7 [4-11]	+22.6 [6-62]*
Total apo B, mg/dL	81.2 (14.1)	79.8 (14.0)	−1.6 (6.4)*	79.2 (15.6)	−2.7 (6.7)*
Apo B-48, mg/dL	0.51 [0.4-0.8]	1.24 [0.9-1.4]	+121.3 [90-163]‡	1.42 [0.9-2.0]	+147.2 [94-231]‡

Data for 12 subjects are means (SD) or median [interquartile range]. 4 h: 4 hours after breakfast; 8 h: 8 hours after breakfast and 4 hours after lunch.

* *P* = not significant.

† *P* < .05.

‡ *P* < .001.

Table 3
Mean values, percentage change, and *P* value at fasting and postprandial in subjects with obesity

Variable	Fasting	Postprandial	Difference	% Change
TC, mg/dL	221.2 (36.6)	220.6 (36.8)	−0.6 (17.7)	−0.02 (7.5)*
TG, mg/dL	133 [104-198]	253 [176-366]	+108 [62-169]	+72.7 [43-105]‡
HDL-C, mg/dL	42.5 (12.9)	34.8 (13.4)	−7.8 (5.3)	−19.9 (15.4)‡
TC/HDL-C ratio	5.0 [4.2-6.7]	6.6 [4.8-8.2]	+0.9 [0.4-2.3]	+17.0 [9.5-40]‡
Non–HDL-C, mg/dL	178.7 (39.4)	185.8 (40.9)	−7.1 (18.7)	+4.5 (10.6)†
LDL-C, mg/dL	135.6 (27.2)	118.4 (28.0)	−17.2 (16.4)	−12.6 (12.3)‡
RemL-C, mg/dL	10.8 [7-15]	18.8 [12-26]	+6.9 [4-12]	+58.1 [33-96]‡
Total apo B, mg/dL	99.5 (20.5)	98.2 (21.1)	−1.4 (9.8)	−1.0 (10.4)*
Apo B-48, mg/dL	0.82 [0.53-1.41]	1.84 [1.21-2.98]	+1.03 [0.57-1.45]	+105.9 [56-200]‡

Data for 63 subjects are means (SD) or median [interquartile range].
* *P* = not significant.
† *P* < .05.
‡ *P* < .001.

hemoglobin values of at least 106 mg/dL and bilirubin values of at least 10 mg/dL. To further validate this assay, we measured apo B-48 in whole plasma and the TRL fraction (after subjecting plasma to ultracentrifugation at its own density of 1.006 g/mL for 18 hours) in the first study as mentioned above and previously described [8]. For the apo B-48 ELISA in our laboratory, intra- and interassay coefficients of variation were 0.96% and 2.17%, respectively. In addition, on all TRL samples, total apo B was measured by ELISA; and the relative amounts of apo B-100 and apo B-48 were assessed by densitometric scanning as previously described to calculate TRL apo B-48 levels as described [16].

Direct LDL-C, HDL cholesterol (HDL-C), and remnant lipoprotein cholesterol (RemL-C) were measured using assays obtained from Kyowa Medex (Tokyo, Japan) as previously described [17-20]. Total cholesterol (TC) and TG levels were measured using kits obtained from Roche Diagnostics (Indianapolis, IN). Our laboratory participates in the Lipid Research Clinic Centers for Disease Control Lipid Standardization Program (Atlanta, GA). All lipid assays were measured on a Hitachi 911 analyzer (Hitachi Inc, Tokyo, Japan). The direct LDL-C assay had intra- and interassay coefficients of variation of 0.73% and 1.13%, respectively, whereas for the direct HDL-C assay, these values were 0.82% and 0.76%. For RemL-C, the values were 1.55% and 3.79%; for the TG assay, the values were 1.44% and 1.17%; and for the TC assay, the values were 1.7%, and 1.07%, respectively.

2.3. Statistical analysis

Descriptive statistics including means (standard deviations) and median values [interquartile ranges] for continuous variables and proportions for categorical variables were computed for all studies. In the first 2 studies involving 9 subjects with combined hyperlipidemia and 12 healthy subjects, the Wilcoxon signed rank test was used to assess the statistical significance of changes of all parameters comparing fasting and postprandial values. Two-sample *t* tests were also used to assess continuous variables using SAS statistical package version 9.1 (SAS Institute, Cary, NC).

3. Results

In the first study in 9 subjects with combined hyperlipidemia, plasma and TRL cholesterol, TG, and apo B-48 values in the fed state (small frequent hourly feeding; time points 0, 3, and 6 hours as part of a kinetic study while on placebo) are presented (Table 1). In this analysis, 16% of cholesterol, 74% of TG, and 64% of apo B-48 in plasma were in TRL. These 9 subjects were studied on placebo as well as on atorvastatin at doses of 20 mg/d and 80 mg/d as described [8]. The mean plasma apo B-48 value was 1.93 mg/dL (SD, 0.73 mg/dL) on placebo and 80 mg/d on atorvastatin; this value decreased to 1.26 mg/dL (SD, 0.38 mg/dL), representing a decrease of 34.8% ($P < .01$). The measured TRL mean apo B-48 value as assessed by ELISA was 1.35 mg/dL (SD, 0.37 mg/dL) on placebo and was 38.5% lower on atorvastatin 80 mg/dL (SD, 0.27 mg/dL). Seventy-five TRL (density <1.006 g/mL) samples from 9 subjects using all TRL samples from placebo and atorvastatin studies had TRL apo B-48 measured by ELISA, as well as having the TRL samples subjected to measurement of total apo B by ELISA and gel electrophoresis for the separation of apo B-100 and apo B-48, followed by gel scanning. In this analysis, the mean TRL apo B-48 value was 1.01 mg/dL (SD, 0.43 mg/dL) as measured by ELISA and 0.50 mg/dL (SD, 0.20 mg/dL) as assessed by gel scanning. The correlation coefficient or *r* value between the 2 methods was 0.82 ($P < .001$, Fig. 1). Therefore, although the 2 methods correlate well, the gel method yielded TRL apo B-48 concentrations that were about 50% lower than ELISA values, indicating that a correction factor of 2.02 would need to be applied to prior kinetic studies where TRL apo B-48 levels were assessed by gel scanning. In addition, we ran correlations between apo B-48 and TG combining the 0, 3, and 6 time points all drawn in the fed state. Plasma apo B-48 was significantly correlated with plasma TG levels

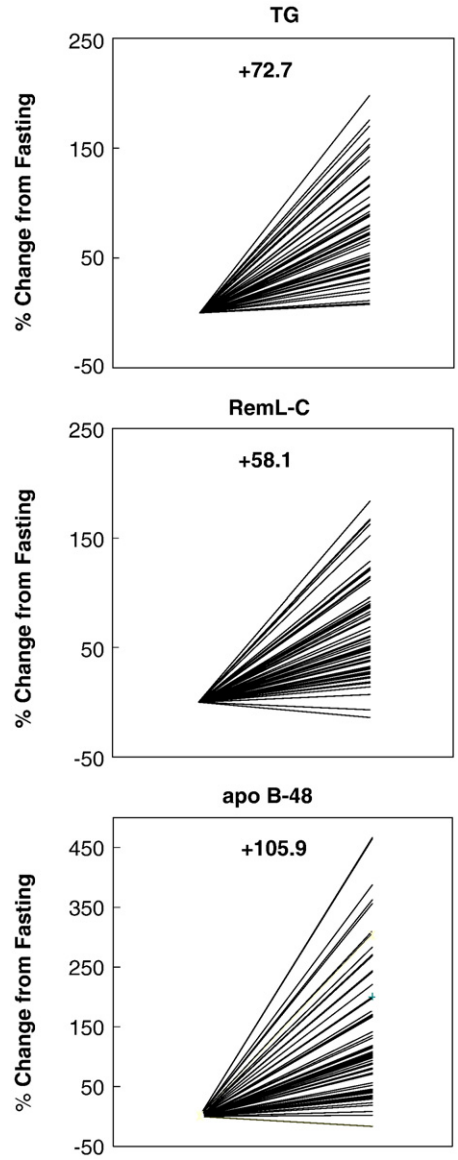

Fig. 3. Variability in postprandial responses of TG, RemL-C, and apo B-48 levels with obese subjects are shown.

($r = 0.77$, $P < .001$) and TRL apo B-48 with TRL TG levels ($P = .68$, $P < .001$).

In the second study (Table 2), data on lipid, total apo B, and apo B-48 values on 12 healthy subjects sampled in the fasting and fed states are provided. In this study, median TG values increased by 50% 4 hours after a standard breakfast and by 66% 8 hours after a standard breakfast and 4 hours after a standard lunch. Even greater increases in apo B-48 levels of 121% and 147% were noted, although total apo B, TC, and non–HDL-C changed very little. Remnant lipoprotein cholesterol increased by 33% and 23%, whereas LDL-C and HDL-C levels decreased modestly by 6% and 9%. The marked variability in postprandial changes in TG, RemL-C, and apo B-48 levels as compared with fasting values is shown in Fig. 2. We also ran correlation coefficients examining the association between the absolute change in apo B-48, remnant cholesterol, and TG values. The change in plasma apo B-48 levels was significantly associated with the change in plasma TG, but not the change in remnant cholesterol levels at 4 hours ($r = 0.59$, $P < .05$ and $r = 0.39$, $P = .21$, respectively). At 8 hours, there was a significant correlation between the change in apo B-48 and the change in both TG and remnant cholesterol levels ($r = 0.89$, $P < .001$ and $r = 0.93$, $P < .001$).

In the third study, 63 overweight and obese subjects were studied using a similar design as in the second study except that these subjects were sampled in the fasting state and 8 hours after breakfast and 4 hours after lunch while on an isocaloric average American diet. In this setting in these individuals, TG levels increased by 73%, RemL-C levels by 58%, and apo B-48 levels by 106%. As in the prior study, TC, non–HDL-C, and total apo B changed very little, whereas direct LDL-C decreased by 13% and direct HDL-C decreased by 20%. As in study 2, there was a marked variability in TG, RemL-C, and apo B-48 postprandial response in these overweight and obese subjects as shown in Fig. 3. In these 63 subjects, the change (postprandial − fasting) in plasma apo B-48 was significantly associated with the change in TG and remnant cholesterol levels ($r = 0.51$, $P < .001$ and $r = 0.44$, $P < .01$, respectively).

In the fourth and final study, we compared data obtained in 270 hyperlipidemic subjects with those in 12 healthy subjects based on plasma samples obtained after a 12-hour overnight fast. As can clearly be seen in Table 4, hyperlipidemic subjects had markedly higher TC, TG, non–HDL-C, LDL-C, RemL-C, and total apo B of about 50% or more, whereas the median apo B-48 value was 37% higher than in controls. In both healthy and hyperlipidemic subjects, only 0.6% and 0.4% of total plasma apo B were present as apo B-48. When all 282 subjects were combined, fasting apo B-48 levels were significantly associated with fasting TG and RemL-C levels ($r = 0.54$, $P < .001$ and $r = 0.54$, $P < .001$, respectively).

Table 4
Mean value and *P* values at fasting with hyperlipidemic cases and controls

Variable	Controls	Hyperlipidemic cases	*P* value for differences
TC, mg/dL	184.9 (21.4)	280.5 (28.7)	<.001
TG, mg/dL	90.5 [71-117]	170 [126-222]	<.001
HDL-C, mg/dL	52.4 (8.6)	47.9 (14.2)	.2739
TC/HDL-C ratio	3.6 (0.8)	6.4 (1.9)	<.001
Non–HDL-C, mg/dL	132.5 (23.9)	232.7 (29.8)	<.001
LDL-C, mg/dL	111.8 (20.8)	188.8 (24.7)	<.001
RemL-C, mg/dL	5.4 [3-7]	12.1 [8-17]	<.001
Total apo B, mg/dL	81.2 (14.1)	174.5 (24.0)	<.001
Apo B-48, mg/dL	0.51 [0.37-0.76]	0.70 [0.50-1.00]	.0648

Data for 270 subjects with hyperlipidemic cases and 12 control subjects are means (SD) or median [interquartile range].

4. Discussion

The measurement of apo B-48 has been hampered by the availability of specific immunoassays to measure the levels of this apolipoprotein in plasma, serum, or isolated lipoproteins. Sakai et al [21] in 2003 have reported on results obtained with an apo B-48 ELISA developed with the use of monoclonal antibodies. This assay had coefficients of variation of approximately 10%, and the investigators reported median serum apo B-48 levels of 0.52 mg/dL in healthy subjects and significantly higher values in patients with severe hypertriglyceridemia (>400 mg/dL) [21]. They also reported about a 2-fold increase in serum apo B-48 levels after a fat loading test [21]. This area was extensively reviewed by Jackson and Williams [22] in 2004; and they reported on a total of 16 studies where TRL apo B-48 levels were assessed by gel electrophoresis, staining with Coomassie blue, and gel scanning. Using these methods, concentrations ranging from trace values to 0.813 mg/dL were reported. In these studies, postprandial increases of 150% to 900% were reported. In addition, they report on 6 studies using gel electrophoresis followed by Western blotting with specific apo B antiserum; and here, values of 0.70 to 6.40 mg/dL were reported, mainly in TRL. In 2 of these studies, 150% increases in apo B-48 were reported postprandially. These authors reviewed 11 studies using ELISAs in plasma, serum, and TRL. They report levels that range from 0.0008 to 0.53 mg/dL in plasma or serum and from 0.02 to 0.05 mg/dL in 2 studies in TRL. They also report postprandial increases of 140% to 450% in plasma/serum apo B-48 and 220% to 1200% in various TRL fractions [22]. The authors concluded that there was a great deal of variability in the results obtained using various methods of measuring apo B-48 in plasma, serum, or the TRL fraction.

In the studies presented here, we document that the results obtained with the apo B-48 ELISA that we have tested correlated well with a TRL gel scanning method coupled with a total apo B ELISA. However, we also noted that the values obtained with the ELISA assay were about 2-fold higher than those obtained by gel scanning. Because the apo B-48 ELISA that we tested was calibrated using recombinant apo B-48 antigen, we have greater confidence that the ELISA values are more accurate than the values obtained by the gel scanning methodology. Of note is that our median plasma apo B-48 values were almost identical to those reported by Sakai et al [21]. Moreover, these investigators also reported about a 2-fold increase in serum apo B-48 levels with feeding.

In our studies with the apo B-48 ELISA that we tested, we noted excellent sensitivity and specificity, with no detectable cross-reactivity with apo B-100, as reported previously [14,15]. Presumably, the monoclonal antibody that was developed for this assay is specific for apo B-48 on lipoprotein particles and therefore may well be conformation specific because apo B-48 contains the initial 48% amino acid found in apo B-100. This possibility could potentially affect the ability to reliably measure apo B-48 in different-sized TRLs. Nevertheless, we do feel that this methodology is far more accurate than gel-based technology that relies on the chromogenicity of apo B-48 vs other lipoproteins within TRL. In addition, we documented very reasonable correlations between plasma and TRL apo B-48 and TG, as well as between fasting and postprandial plasma apo B-48 values. These data suggest that enrichment of TRL with TG in the fed state does not significantly affect the measurement of apo B-48 with this ELISA.

We documented that about two thirds of plasma apo B-48 is found within TRL, similar to TG and consistent with data reported by Campos et al [23]. Moreover, we noted median fasting apo B-48 values in plasma of 0.51 mg/dL; and these values increased by 121% 4 hours after a standard breakfast and by 147% 4 hours after a standard lunch (and 8 hours after this same breakfast). These relative increases were substantially greater that those observed for TG (50% and 66%, respectively). We noted in obese subjects that median apo B-48 levels were 0.82 mg/dL and that these values increased by 106% 8 hours after a standard breakfast and 4 hours after a standard lunch, with corresponding TG increase of 73%. Finally, in hyperlipidemic subjects, median plasma apo B-48 values were 0.70 mg/dL, 37% higher than control values, whereas total apo B values were 115% higher. These latter data indicate that in the fasting state hyperlipidemic subjects with cholesterol and TG values of 281 mg/dL and 170 mg/dL mainly have an increase in liver-derived very low-density lipoprotein and LDL, rather than lipoprotein of intestinal origin. In healthy subjects, apo B-48 comprised about 0.6% of total plasma apo B, whereas in subjects selected for elevated lipids, this percentage decreased to 0.4%. Our data indicate that specific apo B-48 immunoassays are now available for the detection of abnormalities of intestinal lipoproteins in plasma or serum.

It has been reported that subjects with coronary heart disease have elevations in remnant lipoproteins as compared with controls, but remnant lipoproteins are composed of both intestinal and liver-derived plasma lipoproteins [24-27]. The metabolisms of TRL apo B-48 and apo B-100 are similar, except that it is TRL apo B-48 fractional catabolic rate that is inversely related to HDL apo A-I fractional catabolic rate [27,28]. In addition, it is now clearly known that statins and niacin can lower both TRL apo B-100 and apo B-48 by enhancing their fractional catabolism. In our view, lipoproteins of both liver and intestinal origin are atherogenic; and apo B-48 is the only specific marker of intestinal lipoproteins [29-34].

Acknowledgment

S Otokozawa and M Ai were supported by research fellowships from Kyowa Medex, Tokyo, Japan, and Denka Seiken, Tokyo Japan, respectively. BF Asztalos and EJ Schaefer were supported by grants R01 HL-60935, HL

74753, and PO50HL083813 from the National Institutes of Health and contract 53-3K–06 from the United States Department of Agriculture Research Service.

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