Postprandial lipemia in subjects with hypobetalipoproteinemia and a single intestinal allele for apoB-48

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Abstract Hypobetalipoproteinemia in many kindreds is associated with truncated forms of apoB-100. Mutations of the apoB gene specifying more than 20 different carboxyl terminal truncations of apoB have been identified ranging in length from apoB-2 to apoB-89. Truncations longer than apoB-48 appear to be secreted only by liver, while truncations shorter than apoB-48 are secreted by liver as well as intestine. Thus, intestines of subjects heterozygous for truncations > apoB-48 contain two alleles producing apoB-48, while intestines of heterozygotes with truncations < apoB-48 contain only one allele producing apoB-48. Our aims were to assess whether intestinal fat absorption differed from normal in subjects with apoB-truncation-associated hypobetalipoproteinemia and whether fat absorption in heterozygotes with apoB < 48 differed from heterozygotes with apoB > 48. Ten subjects heterozygous for apoB > 48 (apoBs -89, -75, -54, -52), six heterozygous for apoB < 48 (apoBs -46, -40, -31) and a group of 16 controls matched for age, sex, body mass index characteristics, and eating similar diets were given identical fat meals containing vitamin A. Plasma triglycerides in whole plasma and retinyl palmitate in chylomicron and non-chylomicron (remnant) fractions were analyzed at zero time and over the next 14 hours. Fasting vitamins A and E also were quantified. Fasting plasma levels of vitamin E were lower in heterozygotes (536 ± 198 mg/l for apoB > 48 vs. 372 ± 155 for apoB < 48) versus controls (1162 ± 441), but were not different when corrected for differences in LDL-C. Plasma vitamin A levels (uncorrected) were not different. Meal responses were characterized in terms of peak concentrations and areas under the curves (after subtraction of minimum points). These indices of fat absorption were comparable in all apoB phenotype groups suggesting that one allele specifying the intestinal production of apoB-48 is sufficient for normal fat absorption. **—Averna, M., R. L. Seip, K. Mankowitz, and G. Schonfeld.** Postprandial lipemia in subjects with hypobetalipoproteinemia and a single intestinal allele for apoB-48. *J. Lipid Res.* 1993. **34:** 1957–1967.

Supplementary key words hypobetalipoproteinemia • truncated apoB • vitamin A • vitamin E • retinyl palmitate • triglycerides • chylomicrons • chylomicron remnants

Familial hypobetalipoproteinemia (FHB) is an autosomal co-dominant disorder characterized by very low plasma levels of total- and LDL-cholesterol and apolipoprotein B (apoB). Heterozygotes for the condition are usually asymptomatic, while homozygotes may suffer from sequelae of intestinal fat malabsorption (1). In some kindreds this syndrome may cosegregate with truncated forms of apoB-100. To date, over 20 different truncations have been identified (2). In other kindreds no truncations of apoB are identifiable. The hypobetalipoproteinemias should be distinguished from both abetalipoproteinemia and chylomicron retention disease, both of which are autosomal recessive conditions characterized by severe fat malabsorption detectable in infancy (3–5).

Two natural forms of apoB are present in plasma: apoB-100 and apoB-48. The former contains 4536 amino acids, the latter 2152. Both are encoded by the same gene, but are products of translation of two different mRNAs that are identical except at nucleotide position 6666, where a unique editing mechanism changes nucleotide C (for apoB-100 mRNA) to U (for apoB-48 mRNA). The base substitution introduces a STOP codon at position 6666, corresponding to amino acid 2153. This results in premature termination of translation and the secretion of apoB molecules with 52% of their carboxyl terminal portions missing (6). In humans, apoB-100 is secreted only by liver and in plasma it is associated with VLDL, while apoB-48 is secreted only by enterocytes and in plasma it is associated with chylomicrons. In homozygous abetalipoproteinemia neither form of apoB is secreted, possibly due to the absence of microsomal triglyceride transfer

Abbreviations: FHB, familial hypobetalipoproteinemia; LDL, low density lipoproteins; VLDL, very low density lipoproteins; RP, retinyl palmitate; HDL, high density lipoproteins; TRL, triglyceride-rich lipoproteins; CM, chylomicrons; CMR, chylomicron remnant; BMI, body mass index; TG, triglyceride; AUC, area under the curve; C, cholesterol.

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protein, resulting in defective assembly of both VLDL-apoB-100 and chylomicron-apoB-48 with triglycerides (7). The molecular defect(s) in chylomicron retention disease is (are) unknown, but chylomicron-like particles are retained in enterocytes (3).

It is not clear to what extent the transport of lipids by truncated forms of apoB is abnormal because for most truncations, rates of secretion and the compositions of the lipoproteins with which they are associated are not known. However, it is highly probable that truncations shorter than apoB-48 are secreted by both liver and intestine while truncations longer than apoB-48 are secreted only by liver (8). Thus, heterozygotes for the former have only one normally functioning allele for apoB-48 in their enterocytes, and heterozygotes for longer truncations have two functioning alleles producing apoB-48. Therefore, we hypothesized that intestinal fat absorption may be defective in apoB < 48 heterozygotes, while apoB > 48 heterozygotes should have normal fat absorption. To test this hypothesis, 17 subjects with various truncations and 16 matched normal controls were given a vitamin A-containing fat meal and the time courses of appearance and disappearance of retinyl palmitate in the chylomicron and remnant fractions were followed, along with the concomitant fluctuations in plasma triglycerides, apoB-100, apoB-48, and the apoB-truncations. In addition, to evaluate the plasma levels of fat-soluble vitamins, fasting plasma retinol and vitamin E levels in plasma were also determined.

MATERIALS AND METHODS

Subjects

Sixteen simple heterozygotes and one compound heterozygote for familial hypobetalipoproteinemia having different truncated forms of apoB were studied. All the subjects belonged to kindreds with FHB with truncations previously detected and/or studied at Washington University Lipid Research Center, St. Louis, MO (9–15). ApoB heterozygosities of the 17 subjects were: 31/100 = 1; 37/100 = 2; 40/100 = 2; 46/100 = 1; 52/100 = 2; 54.8/100 = 4; 75/100 = 2; 89/100 = 2 and 89/40 = 1. Sixteen normolipidemic healthy subjects were selected as control group for evaluation of fasting levels and postprandial lipid responses. None had endocrine, liver, or renal diseases and none were taking medications. Attempts were made to match controls to FHB subjects by age, gender, and body mass index. All subjects gave informed consent to a protocol approved by the Human Studies Committee of Washington University School of Medicine. Clinical characteristics of FHB subjects and controls, including apoE phenotypes, are shown in **Table 1.** Dietary habits of FHB subjects and controls were recorded using 3-day diaries. The patients were assisted in the diary compilation by two experienced dietitians. The average dietary intake was calculated by a computer program (Minnesota Nutrition Data System, University of Minnesota, Minneapolis, MN) and listed in **Table 2.**

Vitamin A fat-loading test

After a 12-h fast, subjects were given a fatty meal enriched with 60,000 units of vitamin A per m^2 of body surface area. The fatty meal consisted of one packet of a special formulated product (Carnation Instant Breakfast, dry mix, chocolate), 2 cups of skim milk, and 100 g corn oil, yielding 1185 kCal, 77% from fat, 16% from carbohydrates, 8% from protein, with a P:S ratio of 4. After the meal the subjects ate no calories for 14 h, but were allowed to drink water or noncaloric drinks. Blood samples were drawn before the meal, every hour until the 6th h and every 2 h until the 14th h.

Lipoprotein separations

Venous sample tubes, wrapped in foil, and containing sodium EDTA were immediately centrifuged at 1,500 *g* for 15 min, and 2.5 ml of plasma was transferred into 0.5 × 2.5 in polyallomer tubes and overlayered with 0.15 M NaCl, 1 mM EDTA (pH 7.4, d 1.006 g/ml). Tubes were ultracentrifuged for 20 min at 20,000 rpm in the outer row of a 50.4 rotor (Beckman Instruments, Fullerton, CA). Chylomicrons, contained in the 1.25-ml top layer, were removed by aspiration after cutting the tubes and the infranatant was centrifuged at a density of 1.006 g/ml for 18 h at 35,000 rpm in the same rotor. A volume of 1.25 ml containing the non-chylomicron fraction (also referred

TABLE 1. Clinical characteristics

Subjects	Age	Height	Weight	BMI	Smoking Habit	ApoE Phenotype and Genotype
	yr	*cm*	*kg*			
Controls						
Males (n = 10)	56.1 ± 18.0	174.7 ± 6.6	78.6 ± 17.6	25.6 ± 4.7	2/10	3/3 = 7, 3/4 = 1, 4/4 = 1, nd = 1
Females (n = 6)	45.3 ± 11.7	163.8 ± 5.5	67.9 ± 9.0	25.2 ± 2.2	2/6	3/3 = 2, 3/4 = 3, 4/4 = 1
Subjects with truncated apoB						
Males (n = 11)	46.7 ± 17.6	178.0 ± 5.0	83.7 ± 11.5	26.3 ± 2.9	6/11	3/3 = 7, 2/2 = 2, 3/4 = 1, nd = 1
Females (n = 5)	44.2 ± 13.4	169.7 ± 6.1	88.0 ± 30.2	30.6 ± 10.3	1/6	3/3 = 2, 3/4 = 2, 4/4 = 1

Results are means ± SD; BMI, body mass index, kg/m^2; nd, analysis not done.

TABLE 2. Dietary intake

	Controls	ApoB Truncations
Calories	2034 ± 585	2209 ± 748
Proteins g/d (% of calories)	86 ± 31 (17 ± 2)	85 ± 31 (16 ± 2)
Carbohydrates g/d (% of calories)	227 ± 101 (46 ± 8)	252 ± 14 (47 ± 10)
Lipids g/d (% of calories)	76 ± 16 (35 ± 7)	88 ± 39 (36 ± 9)
Ethanol (% of calories)	2.8 ± 3.5	3.2 ± 4.7
Cholesterol mg/d	200 ± 93	300 ± 166
Calories from fatty acids (g/d)		
Saturated	24 ± 7	32 ± 124
Monounsaturated	29 ± 7	34 ± 16
Polyunsaturated	16 ± 7	15 ± 9
P/S	0.6 ± 0.3	0.5 ± 0.1

Based on 3-day diaries; results are means ± SD.

to as remnants) was removed from the top of the tube. All operations were done in subdued light. Chylomicron and remnant fractions were stored at −70°C until assayed for retinyl palmitate.

Retinyl palmitate assay

The retinyl palmitate (RP) content of chylomicrons and remnant fractions was assayed using a modification of a method previously described (16). Briefly, to chylomicrons and remnant fractions was added 50 μl of an internal standard solution (retinyl acetate in methanol) and fractions were extracted by ethanol–hexane–water 3:4:3 (v:v:v). The hexane upper phase was evaporated under nitrogen, redissolved with methanol, and injected onto a HPLC 250 mm × 4.6 mm reversed phase column packed with Econosphere C_{18} 5μ (Alltech Associates, Inc., Deerfield, IL). Methanol–heptane 95:5 mixture was used as mobile phase at a flow rate of 1 ml/min in a Varian Model 5000 liquid chromatograph equipped with a 3392A integrator (Hewlett-Packard, Avondale, PA) and a M001.728 autosampler (Micrometrics, Norcross, GA). The effluent was monitored at 340 nm. The peak of retinyl palmitate was identified by comparing its retention time with a purified standard (Sigma, St. Louis, MO) and the area was quantitated by the area ratio method (17) using retinyl acetate as internal standard. Here too, all operations were performed in subdued light.

Lipid and apolipoprotein analyses

Cholesterol and glycerol-blanked triglycerides were measured using Miles-Technicon (Tarrytown, NY) enzymatic reagents in a Technicon RA-1000 AutoAnalyzer. HDL-cholesterol was measured by precipitation method using dextran-sulfate-Mg^{2+} (MW 50,000 Genzyme, Boston, MA). ApoA-I and apoB were determined by nephelometric immunoassays (Behring, Summerville, NJ). Lipids and apolipoproteins were determined in the Washington University Lipid Research Center Core Laboratory, using commercial kits (Technicon, Tarrytown, NY) and protocols of the Lipid Research Clinics (18).

Quantitation of apoB-100, apoB-48, and truncated apoB by SDS gradient gel electrophoresis

Two ml of plasma for each timepoint of the oral fat test was ultracentrifuged in 0.5 × 2.5 in tubes placed in the outer row of a 50.4 rotor (Beckman, Fullerton, CA) at 35,000 rpm for 18 h. The 1.25-ml top layer containing the triglyceride-rich lipoproteins (TRL) (chylomicrons, remnants, plus VLDL) was removed and assayed for protein (19). An aliquot containing 30 μg of TRL protein was lyophilized, reduced in SDS-sample buffer (2% β-mercaptoethanol, 1% SDS, 0.3% bromophenol blue, 0.5 M Tris, pH 6.8, and glycerol, 10%) for 2 min at 90°C, and analyzed on polyacrylamide 3%–6% gradient sodium dodecyl sulfate (SDS) polyacrylamide gel as previously described (20). Gels were run at 90 mA until the dye front was 0.5 cm from the bottom of the plate. Gels were stained for 2 h with 0.2% Coomassie Brilliant Blue and destained with 10% acetic acid. The different forms of apoB were identified by comparison with known molecular weight standards (Pharmacia, Piscataway, NJ). Gels were scanned using a computer image analysis program (JAVA, Jandel Scientific, Corte Madera, CA) which analyses both the intensity and the area of the bands. The quantitation of each of the apoB at different timepoints was performed as previously described (21) according to the formula:

$$\frac{X\ (a.u.) \times C_T\ (mg/dl) \times 100}{Y\ (a.u.) \times C_0\ (mg/dl)}$$

where X = intensity of the apolipoprotein band at timepoint after the fat meal; Y = intensity of apolipoprotein band at zero time; a.u. = arbitrary units; C_T = TRL total protein concentration after the fat meal; C_0 = TRL total protein concentrations in plasma at zero time. The results were expressed in terms of relative increase of each apoB form at each timepoint.

Fat-soluble vitamins assay

Fasting plasma levels of retinol (vitamin A) and α-tocopherol (vitamin E) were determined in 41 healthy subjects and in 15 FHB subjects (31/100=1, 37/100=2, 40/100=2, 46/100=1, 52/100=2, 54.8/100=2, 75/100=3, 89/100=1, 40/89=1). Vitamin A and E levels were measured according to a modification of the method of Catignani and Bieri (22). Briefly, to 1 ml of plasma were added retinyl acetate and tocopherol acetate as internal standards and plasma was extracted by hexane–water–ethanol 4:3:3. The residue was dried, redissolved in methanol, and injected onto a reverse phase column C_{18} 5 μ (Econosphere by Alltech Associates, Inc., Deerfield, IL) using the HPLC equipment described above. The mobile phase was methanol at a flow rate of 1 ml/min. Retinol and α-tocopherol were identified by comparison with purified standards (Sigma, St. Louis, MO). The plasma concentrations of vitamins A and E were determined by the area ratio method, as previously described (17). The results were expressed in μg/l (vitamin A) and in mg/l (vitamin E). All operations were performed in subdued light.

ApoE genotypes and phenotypes

Both apoE phenotyping and genotyping were performed. ApoE phenotyping of the study subjects was performed by isoelectric focusing of VLDL apolipoproteins using the method of Weidman et al. (23) adapted to a commercially available slab gel system (PhastGel 4-6.5, Pharmacia, Piscataway, NJ). ApoE genotyping analysis (24) was performed on enzymatically amplified *Hhal* restriction fragments (25) obtained from genomic DNA.

Statistical analysis

The following variables were calculated to characterize the postprandial responses of plasma triglycerides, chylomicrons, and chylomicron remnants to the test meal. The variable area under the curve (AUC) was the area between the plasma concentration versus time curve and a baseline drawn parallel to the horizontal axis through the lowest observed concentration (either 0 h, 12 h, or 14 h). This area was calculated by a computer program using the trapezoidal rule. Other variables were the peak concentration, which is the average of the peak and the second highest concentration above baseline, and the peak time, which was the average of the time to peak concentration and the time to the second highest concentration. For apoB gel scanning data, the area under the apoB curve for apoB-48, apoB-100, and truncated forms of apoB was calculated in a similar manner using data points obtained through the first 8 h of sampling following the test meal.

Student's paired and unpaired *t*-tests were used to test for differences. Pearson's product moment correlations and linear regression equations were calculated using SAS Statistical Software (SAS Institute, Cary, NC). For comparison of triglyceride area under the curve in truncation subjects versus controls, analysis of covariance was performed using the SAS GLM procedure, using fasting plasma triglyceride concentration as the covariate. Data storage and analyses were accomplished using the VAX system housed in the computer facilities of the General Clinical Research Center of Washington University School of Medicine.

RESULTS

There were no significant differences in BMI between FHB subjects and controls (Table 1) and the apoE phenotypes were similarly distributed in the two groups. Dietary intakes of FHB subjects and controls were not different (Table 2). FHB subjects had significantly lower levels of fasting total- and LDL-cholesterol and apoB than controls and FHB females had higher levels of HDL-C, HDL_2-C, and apoA-I than FHB males (**Table 3**). Fasting

TABLE 3. Lipid and apolipoprotein levels in subjects with hypobetalipoproteinemia having truncated forms of apoB and in normolipidemic controls

Subjects	Cholesterol	Triglycerides	LDL-C	Total HDL-C	HDL_2-C	HDL_3-C	ApoA-I	ApoB
				mg/dl				
Controls								
Males (n = 10)	188.7 ± 26.3	123.8 ± 50.7	122.0 ± 22.2	43.3 ± 11.1	6.2 ± 4.9	37.6 ± 8.6	127.9 ± 15.4	101.2 ± 29.4
Females (n = 6)	194.7 ± 27.1	122.0 ± 47.3	123.5 ± 36.3	44.2 ± 4.8	5.8 ± 2.3	36.6 ± 3.2	142.4 ± 21.2	114.4 ± 24.6
Subjects with truncated apoB								
Males (n = 11)	113.9 ± 29.4[c]	85.8 ± 70.6[c]	48.7 ± 23.3[c]	44.2 ± 13.5	8.6 ± 6.5	35.2 ± 8.9	119.8 ± 22.0	46.6 ± 24.9[c]
Females (n = 5)	117.6 ± 26.7[c]	50.7 ± 16.7[c]	46.0 ± 25.7[c]	60.2 ± 9.6[a,d]	18.3 ± 8.1[a,d]	44.0 ± 4.1[a]	157.0 ± 18.3[b]	38.2 ± 20.9[c]

Results are means ± SD.
[a] $P < 0.05$.
[b] $P < 0.02$ males vs. females.
[c] $P < 0.001$.
[d] $P < 0.01$ vs. gender specific controls.
[e] $P < 0.003$.

TABLE 4. Lipids and apolipoproteins of subjects with truncations shorter and longer than apoB-48 controls

ApoB Truncations	Cholesterol	Triglycerides	LDL-C	HDL-C	HDL_2-C	HDL_3-C	ApoA-I	ApoB
				mg/dl				
B>48 (10)	123 ± 20	88 ± 74	52 ± 21	50 ± 14	12 ± 9	38 ± 9	136 ± 23	48 ± 27
B<48 (6)	102 ± 36	60 ± 25	41 ± 27	47 ± 17	11 ± 8	38 ± 10	125 ± 32	42 ± 18

Results are means ± SD. It is not possible to give accurate estimates of total apoB concentrations in molar terms, but approximate estimations were attempted using the following formula:

$$\left[\frac{[\text{total apoB, mg/dl}] \times 0.3}{\text{fractional size of apoB truncation}} + [0.7 \times \text{total apoB, mg/dl}]\right] \times \frac{10}{5.5 \times 10^5 \text{ mg apoB-100/mmol}} \times 1000 = \text{total apoB } (\mu\text{M}).$$

The formula takes into account the fraction of total apoB present in plasma as the apoB truncation estimated as 0.3, based on relative chromagenicities of apoB bands separated by SDS-PAGE, and it corrects the mass concentrations up to the level the truncation would have were it to have the mass of apoB-100. This permits expression of total mass as μM, using 5.5×10^5 as the molecular weight of apoB-100. In an apoB-100 homozygote with an apoB-100 concentration of 100 mg/dl, the μM concentration would be 1.8. Based on the above formula the range of total apoB for apoB<48 would be 0.9–1.8 μM. The analogous values for apoB>48 would be 0.4–2.0 μM.

lipid and apolipoprotein levels of subjects with apoB truncations longer and shorter than apoB-48 were analogous (**Table 4**).

Effect of apoB length on postprandial lipemia

Indices of postprandial response for triglycerides, and chylomicron- and remnant-retinyl palmitate, such as areas under the curve corrected for the lowest recorded value, height of the peak, and peak time, were not significantly different in subjects with hypobetalipoproteinemia, analyzed as a group versus controls (**Table 5**). Neither did the length of the truncated apoB seem to affect any of the indices of postprandial lipemia (**Fig. 1**), i.e., indices were indistinguishable for subjects with truncations longer and shorter for apoB-48. Because individual differences were so great and standard deviations so large, it is possible that small differences between groups could have been missed (β-error). To assess to what extent the individual differences were due to poor reproducibility of the postprandial fat "tolerance" test, the tests were repeated in two controls and one simple heterozygote and the apoB-89/apoB-40 heterozygote 8–10 weeks apart. Areas under the curve for each subject were averaged and percent deviations from the means were calculated for each area. For TG, these ranged from ±6% to ±14% and averaged 10%; for retinyl palmitate the curve range was 1–6.6% and the mean was 3.5%, suggesting that individual variability rather than poor reproducibility of the tests accounted for the heterogeneity of the results. In this regard, it is noted that one female FHB

TABLE 5. Postprandial lipemic response in hypobetalipoproteinemic subjects according to the apoB length

	Triglycerides		
	AUC (mg/dl 14 h)	Peak (mg/dl)	Peak Time (h)
Controls (16)	1536 ± 732	308 ± 138	6.0 ± 1.5
B>48 (10)	1433 ± 769	273 ± 137	5.5 ± 0.9
B<48 (6)	1224 ± 759	229 ± 156	5.6 ± 0.5
	Chylomicrons (Retinyl Palmitate)		
	AUC (μg/dl 14 h)	Peak (μg/dl)	Peak Time (h)
Controls (14)	9185 ± 4082	1328 ± 652	7.2 ± 1.9
B>48 (8)	13137 ± 8628	1568 ± 913	8.6 ± 1.7
B<48 (6)	12447 ± 4564	1591 ± 497	7.6 ± 1.9
	Chylomicron Remnants (Retinyl Palmitate)		
	AUC (μg/dl 14 h)	Peak (μg/dl)	Peak Time (h)
Controls (14)	2576 ± 2183	417 ± 356	8.0 ± 2.8
B>48 (8)	2999 ± 2495	645 ± 348	8.3 ± 2.2
B<48 (6)	1748 ± 1559	279 ± 215	6.7 ± 2.5

Results are means ± SD; ( ), number of subjects; AUC, area under curve with lowest concentration subtracted (see Methods).

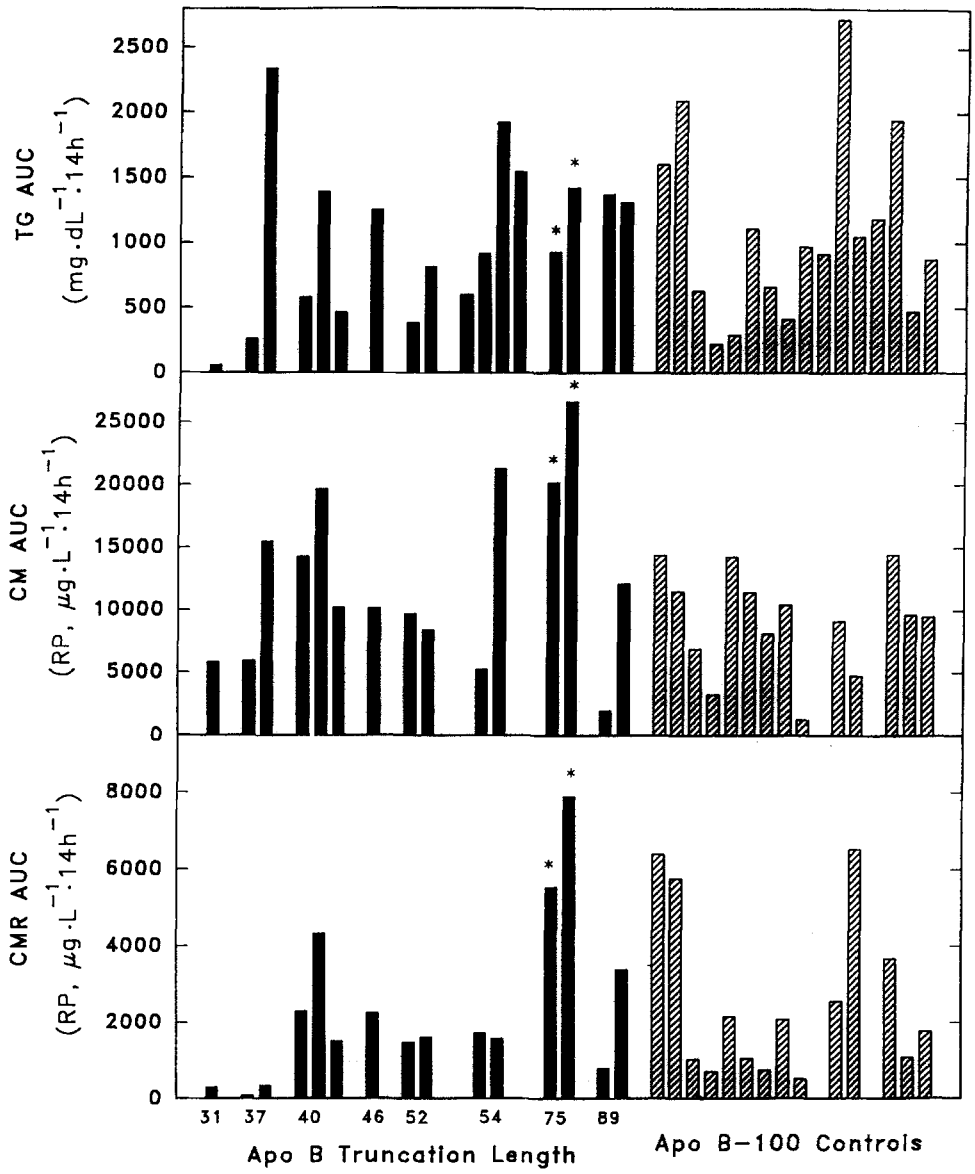

Fig. 1. Plasma triglyceride, chylomicron, and chylomicron remnant responses to a test meal in FHB patients (■, n = 17) and controls (*A*, n = 16). Areas are calculated from plasma concentrations of triglyceride (TG) or retinyl palmitate (RP) measured in the 14 h after ingestion, as described in the text. Abbreviations are: TG AUC, triglyceride area under the curve; CM AUC, chylomicron area under the curve; CMR AUC, chylomicron remnant area under the curve. Asterisks identify subjects with $apoE_2/E_2$ phenotype.

subject (apoB-31), given the test meal twice, vomited on both occasions 12–14 h later and her chylomicron remnant responses were among the lowest. Her data are presented in **Fig. 2,** but not included in the calculations provided in the figures and tables. Her data are included in Table 7.

ApoB-100, apoB-48, and truncated apoB changes during postprandial lipemia

All forms of apoB increased over the first 6–8 h after ingestion of the fat meal (**Table 6**). The relative increases of apoB-100 were smaller than the rises of apoB-48, in normal subjects as well as in subjects with truncations shorter and longer than apoB-48, i.e., relative increase of apoB-48 in all three groups averaged 3–4 times higher than apoB-100. The mean increase of the truncated forms of apoB fell between apoB-48 and apoB-100. There were no significant differences between the shorter and longer truncations and controls.

Effect of age and gender on postprandial lipemia

Some workers have reported that areas under the curve or other indices for fat absorption tend to differ according to age and between the genders (26, 27). Therefore we assessed whether such differences were present among our

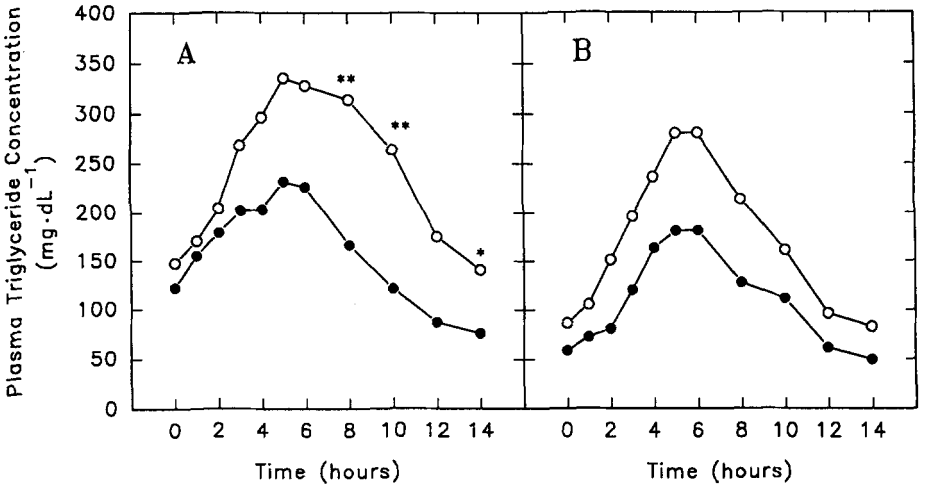

Fig. 2. Plasma triglyceride concentrations after ingestion of a test meal incorporating 100 g corn oil and 60,000 units vitamin A/m² of body surface area in (A), control subjects (n = 10 men, n = 6 women), and (B), in FHB subjects with truncated apoB forms (n = 11 men, n = 5 women). Data for men are shown by the open circles (○), and for women by closed circles (●). Significant differences between genders are indicated by the symbols (*, $P < 0.05$; **, $P < 0.02$).

heterozygotes. Postprandial TG AUC (mg·dl^{-1}·14 h^{-1}) responses in controls were 1135 ± 495 and 1778 ± 765 for females and males, respectively (Fig. 2A) ($P < 0.06$) and in FHB subjects 900 ± 422 and 1561 ± 784 for females and males, respectively (Fig. 2B) ($P < 0.1$). There were no significant differences by gender in the retinyl palmitate curves. There were also no significant differences between subjects younger or older than age 40 years.

Effects of apoE phenotype on postprandial lipemia

Chylomicron RP and non-chylomicron RP responses in FHB subjects with truncated apoB forms and different apoE phenotypes are shown in **Fig. 3.** Mean values of E_3/E_3 subjects were as follows (TG AUC units = mg/dl·14 h^{-1}; chylomicron (CM) and chylomicron remnant (CMR) AUC units = RP, μg·l^{-1}·14 h^{-1}): TG AUC 1154 ± 729 (not shown); CM AUC 10, 748 ± 5078; CMR AUC 1752 ± 1398, n = 9. Mean values of E_2/E_2 subjects were TG AUC 1498 ± 370 (not shown); CM AUC 23,432 ± 4588; CMR AUC 6725 ± 1669, n = 2; and areas for E_4/E_3 heterozygotes were TG AUC 1750 ± 1145 (not shown); CM AUC 12,221 ± 8211; and CMR AUC 1853 ± 360, n = 3. The CMR AUCs for E_2/E_2 subjects were elevated compared to the other groups.

TABLE 6. Changes in the relative concentrations of the various forms of apoB during postprandial lipemia

		FHB Heterozygotes	
	Controls (n = 6)	ApoB > 48 (n = 7)	ApoB < 48 (n = 5)
ApoB-100	2.4 ± 1.3	5.3 ± 7.7	2.1 ± 1.7
ApoB-48	5.2 ± 2.1[a]	9.0 ± 8.4[a]	8.9 ± 6.9
ApoB truncations	N/A	6.7 ± 7.8	4.0 ± 2.7

Results are means ± SD and represent areas under the curves after subtraction of the minimum points. Each data point was obtained by scanning of Coomassie blue-stained 3–6% SDS-gels. For each form of apoB the dye uptake areas of the 0 time points were set at 1 and the dye uptakes of all other areas were related to the 0 time areas. All areas were significantly different from zero; n, number of subjects.

[a] $P < 0.05$ vs. apoB-100 for the same columns.

Effect of fasting triglycerides on postprandial lipemia

Because fasting plasma triglyceride concentration can affect the magnitude of the postprandial lipemic response, we examined the relationship between the former and TG AUC, CM AUC, and CMR AUC within the groups. No relationship was found between fasting triglyceride concentration and CM AUC or CMR AUC for any group. For fasting triglyceride concentration versus TG AUC, correlations were significant (r values ranged from 0.7 to 0.839, $P < 0.01$) (**Fig. 4**). Because fasting triglycerides were significantly lower in FHB subjects versus controls, we compared the TG AUC response after removing the effect of fasting triglycerides (see Statistical analysis, above). The adjusted mean for TG AUC was significantly lower in controls (1237 mg·dl^{-1}·14 h^{-1}) versus FHB subjects (1655 mg·dl^{-1}·14 h^{-1}) ($P < 0.03$).

Correlations

As expected, correlation coefficients (r values) between fasting plasma total- ($r = 0.92$) and LDL-cholesterol ($r = 0.93$) versus apoB were high (n = 32) (all $P < 0.0001$), as were r values for both HDL-cholesterol (n = 28, $r = 0.70$) and HDL_3-cholesterol versus apoA-I (r exceeded 0.75, n = 24, $P < 0.001$). The r values were significant whether controls and FHB heterozygotes were considered separately or together.

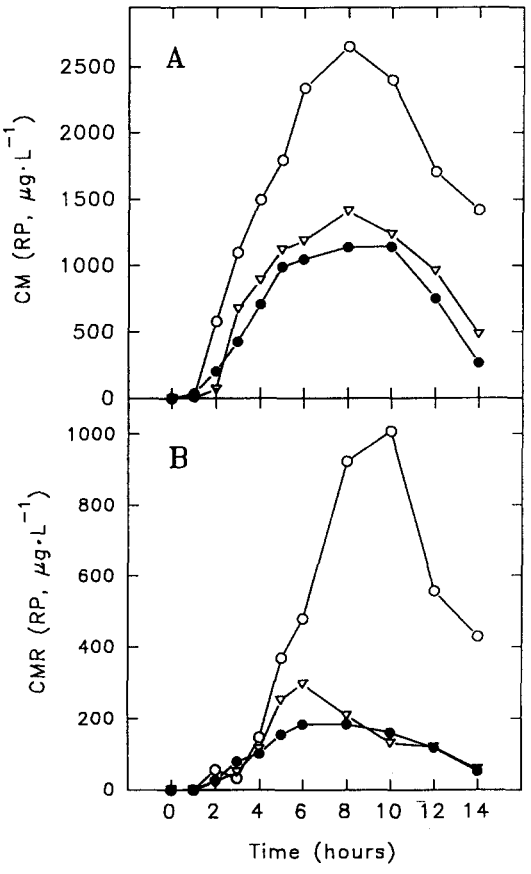

Fig. 3. A: plasma chylomicron (CM) and B: chylomicron remnant (CMR)-retinyl palmitate (RP) responses to the test meal in FHB subjects with E_2/E_2 (○, n = 2), E_3/E_3 (▽, n = 9), and E_4/E_3 (●, n = 3) genotypes for apoE.

In controls, BMI correlated positively with fasting triglyceride ($r = 0.52$, $P < 0.02$). HDL-C and HDL_2-C were inversely correlated with fasting triglycerides ($r = -0.53$, $P < 0.04$; -0.38, $P < 0.05$, respectively), and also with postprandial triglyceride peak concentrations ($r = 0.52$, $P < 0.04$; -0.59, $P < 0.05$). Fasting triglycerides showed a positive correlation with the postprandial triglyceride peaks ($r = 0.91$, $P < 0.0001$) and with postprandial triglyceride AUC ($r = 0.84$, $P < 0.0001$), but were unrelated to either chylomicron or chylomicron remnant RP levels.

Among FHB subjects, HDL-C and HDL_2-C were inversely correlated with fasting triglycerides ($r = -0.55$, $P < 0.03$; $r = -0.54$, $P < 0.06$; n = 13), postprandial triglyceride AUCs ($r = -0.61$, $P < 0.01$; -0.74, $P < 0.004$), and with postprandial triglyceride peaks (HDL-C, $r = -0.60$, $P < 0.01$). Fasting triglycerides were positively correlated to postprandial triglyceride peak concentrations ($r = 0.71$, $P < 0.002$), postprandial triglyceride AUC ($r = 0.79$, $P < 0.0003$) but were unrelated to chylomicron RP AUC ($r = 0.43$, $P < 0.13$). Thus, heterozygosity for apoB truncations did not affect the strengths of the major correlations among the parameters tested.

Fasting plasma levels of vitamin A and vitamin E

Fasting vitamin A and E levels were determined in 41 healthy fasting normolipidemic controls, females and males, ranging in age from 24 to 77 years and 15 FHB subjects with truncated apoB (**Table 7**). Fasting plasma total- and LDL-cholesterol, and total triglyceride concentrations were significantly lower in both groups of FHB subjects. Vitamin A plasma levels did not differ among controls and either heterozygote groups, but vitamin E levels were significantly lower in the FHB subjects. Because of the close relationship between vitamin E and lipids, the vitamin E/total cholesterol ratio and vitamin E/LDL-cholesterol ratio are also presented. The former ratio differs significantly between controls and FHB subjects ($P < 0.0002$), the latter does not. There were no significant differences between the two FHB groups for either vitamin.

DISCUSSION

Heterozygotes for FHB including those persons who are heterozygous for truncated apoBs, e.g., our study subjects, are usually described as asymptomatic (2). The normal development and health status of heterozygotes suggests that functionally severe intestinal lipid malabsorption is not present. Nevertheless, because the intestines of subjects with truncations shorter than apoB-48 probably produce apoB-48 and as well some undetermined amounts of the apoB truncation, whereas the intestines of heterozygotes apoB>48 produce only apoB-48 (8), we hypothesized that differences could exist between the two groups with respect to fat absorption. Therefore, we used the vitamin A oral fat "loading" test, which has been shown by others (28) and now by us to be a reasonably reproducible method for following the postabsorptive behavior of chylomicron and non-chylomicron particles labeled with retinyl palmitate. Although the sizes of the areas under the curves for triglycerides and retinyl palmitate are determined by a variety of known and unknown factors—among the known are gastric emptying rate, fasting triglyceride levels (26, 29–31), diet (28), the composition of the meal (28), age (26, 27), gender (26, 27), lipoprotein lipase activity (16, 29) and apoE phenotype (27, 32)—the fat tolerance test at the present time is the most convenient method for assessing intestinal fat absorption.

Mean values for postprandial indices of intestinal fat absorption are higher in old compared to younger subjects (26, 27, 31), and in men compared to women (26, 27), but reported P values are >0.05. In our controls we too detected modest nonsignificant trends in these direc-

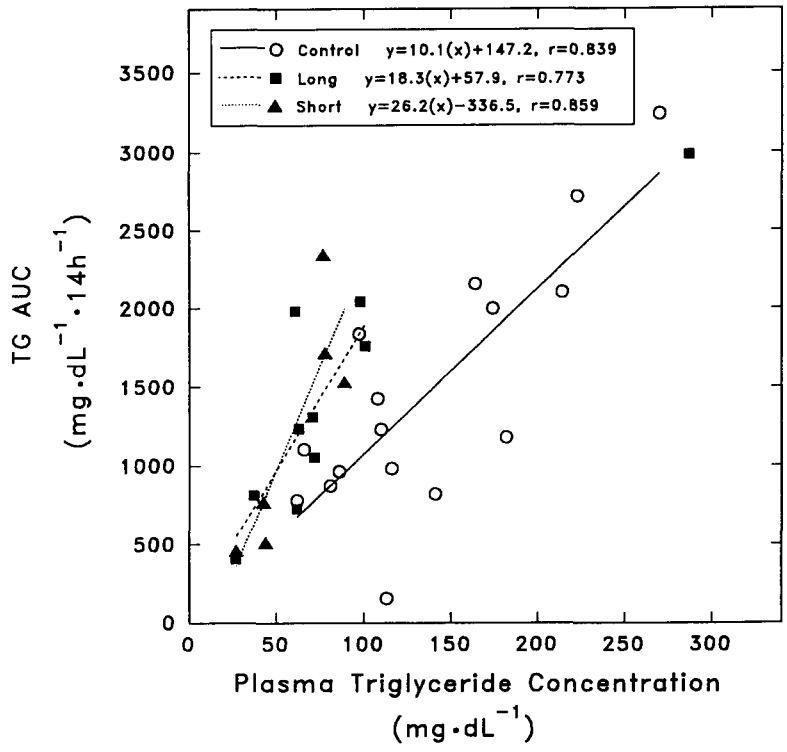

Fig. 4. The relationship between postprandial triglyceride response and fasting plasma triglyceride concentration in controls (○, n = 16), FHB subjects with truncated apoB length >B-48 (■, n = 10, designated as "long"), and FHB subjects with truncated apoB length < B-48 (▲, n = 6, designated as "short"). Linear regression equations were derived for each group by the least squares method. For FHB subjects with apoB length > B-48, regression analysis excluded the subject with fasting triglyceride concentration = 287 mg · dl^{-1}.

tions that were preserved in the FHB group. However, apoE phenotype appears to be a stronger determinant of the chylomicron remnant response than either age or gender (27, 32). Among our FHB subjects, no differences between the E_3 phenotype and E_4/E_3 heterozygotes were detected for postprandial triglyceride, chylomicron, or chylomicron remnant areas, but in the two FHB subjects with phenotype E_2/E_2, chylomicron and chylomicron remnant RP responses were exaggerated compared to FHB subjects with E_3 or E_4/E_3 phenotypes. For chylomicron remnants, this pattern has previously been reported in normolipidemic subjects with either E_2/Ex (where X is 3 or 4) (27) or E_2/E_2 (32) phenotypes. The heightened CMR response seen in our FHB subjects with E_2/E_2 phenotype agrees with the currently accepted hypothesis that suggests that E_2 delays while E_4 enhances chylomicron remnant clearance (27, 32), and provides evidence that the influence of E_2 phenotype on remnant clearance is not altered by the presence of truncated apoB.

Fasting triglyceride levels have also been shown to predict the magnitude of the postprandial lipemic response (26, 29–31), and may modify the effect of apoE phenotype on chylomicron and chylomicron remnant responses (33). We examined the relationship between fasting triglyceride and postprandial triglyceridemia and found that fasting TG and TG AUC were strongly correlated within controls, within FHB with truncations > apoB-48, and within FHB with truncations < apoB-48. Statistical testing indicated that the truncation groups, when combined, responded with a disproportionately higher postprandial triglyceridemic area relative to fasting TG levels, compared to controls. The reasons for this are not clear. It is possible that for any given triglyceride level rates of lipolysis differ between the two groups or that rates of fat absorption are more rapid.

Previously, in normolipidemic subjects, separation of postprandial plasma apoB-48 and apoB-100 forms by SDS gel electrophoresis has shown that both rise and then fall

TABLE 7. Vitamin A and E plasma levels in hypobetalipoproteinemic subjects and in controls

Subjects	Chol	LDL-C	TG	Vit. A	Vit. E	Vit. E/Chol	Vit. E/LDL-Chol
Controls (44)	204 ± 40	128 ± 40	125 ± 53	65 ± 33	1162 ± 441	5.8 ± 2.2	9.9 ± 4.8
Hypobeta B>48 (7)	123 ± 20[a]	52 ± 21[a]	88 ± 74	79 ± 24	536 ± 198[a]	4.4 ± 1.4	11.4 ± 4.4
B<48 (7)	102 ± 36[a]	38 ± 26[a]	60 ± 25[a]	64 ± 29	372 ± 155[a]	3.5 ± 9[b]	12.1 ± 7.8

Results means ± SD; ( ), number of subjects; for cholesterol, LDL-C and TG units are in mg/dl, for vitamin A μg/dl, for vitamin E mg/dl; ratios are mass/mass.

[a] $P < 0.0001$.

[b] $P < 0.01$ vs. controls.

after ingestion of a fat meal. The rise in apoB-48 is significantly higher than that for apoB-100 (21). When apoB gel staining densities for the various forms of apoB (apoB-48 and apoB-100) isolated from plasma sampled over 8 h after the test meal were compared between truncation groups and between each of these two groups and controls, no significant differences were found. Nor were differences detected within truncation groups, when staining densities for apoB-48 or apoB-100 were compared to those of the truncated form. We conclude that all forms of apoB (including apoB-48, apoB-100, and truncated versions of apoB) participate in the postprandial response.

As a further test of fat absorption, fat-soluble vitamins, A and E, were measured in plasma of the FHB and compared to control subject levels. Absolute differences in vitamin E concentrations were large between FHB and controls. The mean levels were 54% lower in FHB subjects with apoB truncations > B-48, and 68% lower in those with apoB truncation length > B-48, compared to controls. Previous reports have suggested that variation in plasma levels are related to LDL-C levels (34–36). In the present study, the absolute differences were also proportionate to LDL-C levels, so that no between-group differences in vitamin A/LDL-C or vitamin E/LDL-C were found.

In summary, our results show that patients heterozygous for FHB and a variety of apoB truncations exhibit a range of responses to a constant vitamin A oral fat loading test that is no different from the responses of a group of controls matched for age, gender, dietary intake, BMI, and apoE phenotypes. Thus, it is unlikely that potentially confounding differences between controls and experimental subjects obscured any differences due to the apoB mutations. Heterozygous truncations shorter or longer than apoB-48 responded normally to an acute fat load. For those heterozygous for apoB < 48, the normal postprandial responses indicate that despite the presence in the intestine of only one normal apoB allele specifying apoB-48, the total amount of apoB-48 produced apparently is sufficient for normal fat absorption to occur. It was impossible to evaluate accurately fat absorption in the apoB-31 heterozygote using oral fat loading because she vomited on two occasions 12–14 h after eating the test meal. Therefore it remains possible that heterozygotes with very short truncations could have some subtle defect in gastric emptying and/or fat absorption.

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