ApoE enhances lipid uptake by macrophages in lipoprotein lipase deficiency during pregnancy

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Abstract A woman with primary lipoprotein lipase (LPL) deficiency developed marked hypertriglyceridemia, pancreatitis, eruptive xanthomas, and unusual palmar xanthomas during pregnancy. Hypotheses to account for the palmar xanthomas were that oxidative modification of triglyceride (TG)-rich lipoproteins occurred due to increased plasma residence time, or that their apolipoprotein E (apoE) content was abnormally elevated. Indices of oxidation of her TG-rich lipoproteins did not support the hypothesis that oxidative changes were a causative factor for her xanthomata. However, degradation of her TG-rich lipoproteins by macrophages was markedly increased (1844 ng/mg protein) during pregnancy as compared to hypertriglyceridemic (with normal LPL) and normotriglyceridemic controls (352 and 126 ng/mg protein, respectively). Post pregnancy the degradation of the subject's TG-rich lipoproteins fell to 289 ng/mg protein. Compositional analysis showed significant enrichment of the particles with apoE (0.97 mass ratio of apoE:apoB during pregnancy, in contrast to 0.38 for normolipidemic controls), and was correlated with the rate of degradation of the TG-rich lipoproteins. Thus, the increased uptake of the TG-rich lipoproteins by macrophages appears to be the result of an unusual enrichment of these lipoproteins with apoE.—**Steinberg, F. M., E. C. Tsai, J. D. Brunzell, and A. Chait.** ApoE enhances lipid uptake by macrophages in lipoprotein lipase deficiency during pregnancy. *J. Lipid Res.* 1996. **37:** 972–984.

Supplementary key words pancreatitis in pregnancy • xanthoma • remnant lipoproteins • lipoprotein oxidation

Primary lipoprotein lipase (LPL) deficiency results from mutations in the LPL gene (1, 2). It is associated with markedly elevated triglyceride levels that can result in pancreatitis and other features of the chylomicronemia syndrome (1). Severe hypertriglyceridemia, eruptive xanthoma, pancreatitis, and even intrauterine fetal death have been associated with pregnancy-induced exacerbation of hypertriglyceridemia in several women with LPL deficiency (3–8). Pancreatitis can be prevented by consumption of a very low fat diet (8). However, consumption of a diet severely restricted in fat during pregnancy can result in difficulty meeting the increased caloric needs and essential fatty acid requirements for normal fetal development. In this report we describe a woman with primary LPL deficiency who developed a severe exacerbation of her hypertriglyceridemia, with resulting pancreatitis, eruptive xanthomas, and unusual palmar xanthomas during the course of her first pregnancy. Palmar xanthomas have been considered to be pathognomonic for remnant removal disease (type III hyperlipoproteinemia) (9) and can be seen in primary biliary cirrhosis (10). This type of xanthoma is believed to result from uptake of remnants of the triglyceride (TG)-rich lipoproteins by macrophages in the skin creases. The individual described in this report had no detectable LPL activity, and therefore would not be expected to be able to form remnant lipoproteins, which are the particles that result from LPL-mediated hydrolysis of the TG-rich lipoproteins.

Two hypotheses were put forth to account for the presence of palmar xanthomas in this pregnant woman with primary LPL deficiency. First, as oxidized LDL is taken up in an unregulated fashion by macrophage scavenger receptors (11), it was proposed that increased oxidative modification of her TG-rich lipoproteins occurred as a result of their increased plasma residence time. A state of physiological hypertriglyceridemia exists in normal pregnancy, reaching approximately 2- to 4-fold basal levels during the third trimester (12, 13). The increased estrogen levels during pregnancy appear to modulate lipid levels by affecting the rate of production of TG-rich lipoproteins (14, 15). Increased triglyceride pool size is associated with reduced fractional clearance

Abbreviations: LPL, lipoprotein lipase; TG, triglyceride; apo, apolipoprotein; DGUC, density gradient ultracentrifugation; TBARS, thiobarbituric acid-reacting substances; LPO, lipid peroxides; AcLDL, acetylated LDL; LRP, LDL-receptor related protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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TABLE 1. Subject characteristics

Subject	Sex	Age	Plasma Chol	Plasma TG	Plasma ApoE
		yrs	*mg/dl*	*mg/dl*	*mg/dl*
LPL-deficient (pregnant)	female	29	284	4518	94
LPL-deficient (post-pregnancy	female	29	122	511	16
HTG-1 (Study 1	male	48	252	728	13
HTG-1 (Study 2)	male	48	232	555	[a]
HTG-2 (Study 1)	female	61	240	745	[a]
Normolipidemic pool (Study 1)	males	27–58	190	108	8
Normolipidemic pool (Study 2)	males	27–58	167	103	4

Chol, cholesterol; TG, triglyceride; Study 1, comparison with primary LPL-deficient subject during pregnancy; Study 2, comparison with primary LPL-deficient subject post pregnancy.
[a]Assay not performed.

rates of triglycerides (16). Thus, the increased production and decreased clearance rates may provide a milieu where oxidative modification of the lipoproteins would be more likely to occur.

An alternative hypothesis was based on the observation that circulating levels of apolipoprotein E (apoE), enrichment with which characterizes remnant lipoproteins, are increased in remnant removal disease (17). ApoE is the primary apolipoprotein responsible for the cellular uptake of chylomicrons, chylomicron remnants, VLDL, and VLDL remnants (18). Although this subject would not be expected to form remnants because of her LPL deficiency, it was hypothesized that the apoE content of her TG-rich lipoproteins might be increased to abnormal levels, leading to increased macrophage uptake in this subject.

Therefore, to determine whether the presence of unusual palmar xanthomas in this LPL-deficient woman during pregnancy might be the result of increased macrophage uptake due to increased oxidative modification of TG-rich LP particles or to increased levels of apoE, both of these hypotheses were evaluated. Products of lipid peroxidation of her TG-rich lipoproteins, and their susceptibility to oxidation ex vivo were determined. The apolipoprotein content of her TG-rich lipoproteins was measured and correlated with the rate of uptake and degradation of the TG-rich particles by human monocyte-derived macrophages in vitro. The results indicate that an increase in the uptake of the TG-rich lipoproteins by macrophages appears to be the result of an unusual enrichment of these lipoproteins with apoE in this individual.

METHODS

Subjects

Fasting plasma was obtained from a 29-year-old female with primary LPL deficiency, two hypertriglyceridemic subjects with plasma triglyceride levels of 500–1000 mg/dl, and from pooled plasma from two pools of six healthy normolipidemic subjects, aged 27–50 years (**Table 1**). All subjects gave informed written consent for the study, which was approved by the Human Subjects Research Committee at the University of Washington.

Clinical course

The 29-year-old female was diagnosed with primary LPL deficiency at the age of 3 months. She is a compound heterozygote for two missense mutations at residues Trp86Arg and His136Arg as determined by single-strand conformation polymorphism and sequencing of the mutant exon (2). LPL mass in plasma and adipose tissue was very low with unstable protein. LPL activity as determined by hydrolysis of a phospholipid-triglyceride emulsion was zero (19). The subject usually had a TG level of < 2000 mg/dl on a self-selected diet of less than 20% of calories as fat. When seen at week 7 of gestation during the course of her first pregnancy, her plasma TG was 396 mg/dl. By week 16, her TG level had risen to 3705 mg/dl. Eruptive xanthomas on the buttocks were noted at week 20 and her plasma TG was in the range of 3000–5000 mg/dl, which persisted even on a diet restricted to < 10% fat (**Fig. 1**). At week 23 she developed intermittent abdominal pain, suspected to be subclinical pancreatitis, that resolved with further dietary fat reduction. By week 27 eruptive xanthoma additionally appeared on her upper arms and medial aspects of thighs. At this time palmar xanthomas also became visible, and a diet of 1–2% fat was instituted. The appearance of palmar xanthoma was surprising, as they are believed to be virtually pathognomonic of remnant removal disease (type III hyperlipoproteinemia) and this subject would not be expected to form remnants by virtue of her LPL deficiency. There was no clinical or biochemical evidence of primary biliary cirrhosis in this subject. Sunflower oil was applied topically (1–2

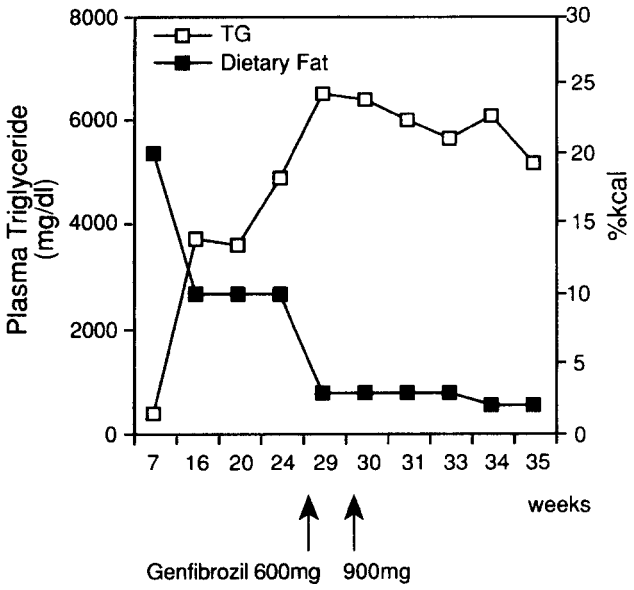

Fig. 1. Plasma triglyceride levels in a primary LPL-deficient subject during pregnancy.

mg/day) as a potentially preventive measure to provide her with a source of essential fatty acids (20). Gemfibrozil (600 mg/day) was begun during week 29 of gestation due to recurrence of abdominal pain and TG levels of approximately 6500 mg/dl, which were refractory to dietary fat restriction. The following week the dose of gemfibrozil was increased to 900 mg/day, which was still less than the usual dose of 1200 mg/day. Thereafter the subject's TG level varied between 5000 and 6000 mg/dl and she was briefly hospitalized during week 32 due to symptoms of TG-induced pancreatitis. This regime continued until labor was induced in week 35 of gestation when a second episode of pancreatitis developed. A healthy baby girl, who had no detectable lipid abnormalities, was delivered. Plasma was obtained from the subject for isolation of TG-rich lipoproteins at week 35 of pregnancy and again at 6 months post-partum.

Materials and reagents

Ficoll-Paque was purchased from Pharmacia (Alameda, CA). RPMI-1640 culture medium and L-glutamine were obtained from BioWhittaker (Walkersville, MD). Primaria 24-well culture plates were purchased from Falcon Labware (Lincoln Park, NJ). Na ^{125}I was purchased from DuPont-New England Nuclear (Boston, MA). All other chemicals and reagents were of analytical grade from Sigma (St. Louis, MO).

Preparation of lipoproteins

A "chylomicron-rich" fraction was isolated and removed by 30 min centrifugation in an SW-41 rotor at 20,000 rpm, 4°C in a Beckman L8-70 ultracentrifuge. The remaining TG-rich lipoproteins were isolated by ultracentrifugation in an SW-41 rotor at 37,000 rpm, for 16 h, 4°C at d 1.006 g/ml and were iodinated with Na ^{125}I using the iodine monochloride method (21). Iodinated TG-rich lipoproteins were used within 2 weeks. Specific activity of the labeled lipoproteins was determined by adjusting protein radioactivity (90–95% of total based on trichloroacetic acid precipitation) by the mass of the protein as measured by the method of Lowry et al. (22).

LDL (d 1.019–1.063 g/ml), for acetylation, was isolated from pooled human plasma (5 mM EDTA final concentration). The LDL was prepared by discontinuous gradient ultracentrifugation in a VTi-50.2 rotor as described previously (23). The lipoproteins were dialyzed against 150 mM NaCl, 1 mM EDTA, pH 7.4, under nitrogen in the dark at 4°C, then sterilized by filtration (0.22 μm) and used within 2 weeks. Acetylated LDL (AcLDL) was prepared according to the method of Basu et al. (24), dialyzed against 150 mM NaCl, 1 mM EDTA, pH 7.4, 4°C overnight, then filter sterilized.

Cells

Human monocytes were isolated by density gradient centrifugation from heparinized blood obtained from fasting normolipidemic subjects, by the method of Böyum (25). Twenty ml of blood was layered over 15 ml Ficoll-Paque and centrifuged at 1,500 rpm for 30 min at 23°C in a Beckman J-6B centrifuge. The mixed mononuclear band was removed by aspiration and the cells were washed twice in RPMI-1640 culture medium containing 6 mM glutamine. The cells were plated at 3×10^5 cells/16-mm dish in the same medium. After 2 h of incubation at 37°C in 95% air/5% CO_2, nonadherent cells were removed by three washes with serum-free medium. The cells were then placed in fresh medium containing 20% autologous serum and were fed twice weekly with the same medium. Monocyte-derived macrophages were used within 7–14 days of plating.

Metabolism of lipoproteins by cells

Cell association and proteolytic degradation of ^{125}I-labeled lipoproteins were measured during incubation of the labeled lipoproteins with cells for 5 h at 37°C. On the day of the experiment, the medium containing autologous serum was removed, the cells were washed twice with RPMI-1640 medium, and different concentrations of ^{125}I-labeled TG-rich lipoprotein were added. Competitors such as AcLDL and activated α_2-macroglobulin were added to some of the experiments. Activated α_2-macroglobulin (2 mg/ml) was prepared by reacting with 25 mM methylamine at pH 8.0 and 25°C for 18 h, after which the mixture was dialyzed overnight

against phosphate-buffered saline (PBS) and filter-sterilized (0.22 μm) before use (26). After incubation of the radiolabeled TG-rich lipoproteins with macrophages for the indicated time interval, the medium was removed and lipoprotein degradation products were assayed in the incubation medium by measurement of trichloroacetic acid-soluble, non-iodide radioactivity (27). Cell-free degradation of the radiolabeled lipoprotein was minimal and was subtracted from total degradation. The cell layer was washed three times with PBS, and extracted by 1 h of incubation at room temperature with 0.5 ml of 0.1 N NaOH for measurement of cell associated radioactivity and protein content by the method of Lowry et al. (22).

Lipoprotein oxidation

The presence of thiobarbituric acid-reacting substances (TBARS) and lipid peroxides (LPO), both indices of lipid peroxidation, was measured in freshly isolated TG-rich lipoproteins. TBARS were analyzed using both 60 μg/ml protein and 300 μg/ml TG from the TG-rich lipoproteins in a volume of 1.5 ml according to Buege and Aust (28) to take into account possible variation in the lipid to protein ratio that can occur with marked hypertriglyceridemia. Lipid peroxides were measured using 60 μg/ml protein and 300 μg/ml TG according to the method of El-Saadani et al. (29). The susceptibility of TG-rich lipoproteins to Cu^{2+}-induced oxidation was assessed by monitoring conjugated diene formation by the method of Esterbauer et al. (30). Ten μM $CuSO_4$ was added to TG-rich lipoproteins to give an adequate rate of oxidation of the lipoprotein. The formation of conjugated dienes was monitored by measurement of A_{234} every 5 min for 8 h. The lag phase and rate of conjugated diene formation were calculated as described (30, 31).

Lipoprotein composition

Cholesterol was assayed using an enzymatic cholesterol kit from Diagnostic Chemicals Ltd. (Oxford, CT), with Preciset cholesterol calibrator standards from Boehringer Mannheim (Indianapolis, IN). Triglycerides were measured enzymatically with reagents and standards from Sigma (St. Louis, MO). Concentrations of apoB in TG-rich lipoproteins were determined nephelometrically as described by Marcovina et al. (32). ApoE concentrations were measured by a competitive radioimmunoassay (33). ApoE phenotypes were determined by isoelectric focusing followed by immunoblotting (34).

Density gradient ultracentrifugation (DGUC)

Plasma (1 ml) was centrifuged in a discontinuous salt gradient at 65,000 rpm for 90 min in a Sorvall 865-B rotor as described by Hokanson et al. (35) in order to optimize resolution of the lipoprotein fractions. Thirty-eight fractions (0.45 ml/tube) were collected from the bottom of the centrifuge tube, which then were assayed for cholesterol content as described previously.

Denaturing polyacrylamide gel electrophoresis (SDS-PAGE)

To estimate content of apoB-48 and B-100 in TG-rich lipoproteins, samples of delipidated lipoproteins from a 30-min centrifugation to isolate "chylomicrons" and from a 16-h centrifugation to isolate "non-chylomicron" TG-rich lipoproteins, were electrophoresed in a 5% polyacrylamide gel after the method of Laemmli (36).

TABLE 2. Oxidation of TG-rich lipoproteins

Subject	TBARS		LPO		CD Lag Time		CD Propagation rate	
	TG[a]	Prot[b]	TG	Prot	TG	Prot	TG	Prot
	nmol MDA/mg		μmol H_2O_2 equivalents/mg		min		Δ A_{234}/h	
LPL-deficient (pregnant)	0.59	3.9	18.3	20.7	318	260	0.32	0.06
LPL-deficient (post-pregnancy)	0.77	2.8	27.0	39.8	160	148	0.50	0.18
HTG-1 (Study 1)	0.62	3.2	3.3	2.5	[c]	[c]	[c]	[c]
HTG-1 (Study 2)	0.85	4.2	16.3	5.2	267	272	0.65	0.70
HTG-2 (Study 1)	1.23	5.6	38.0	12.3	103	95	0.91	0.22
Normolipidemic pool (Study 1)	1.0	1.9	41.7	[c]	157	[c]	0.94	[c]
Normolipidemic pool (Study 2)	0.78	5.9	23.0	26.5	153	150	0.82	0.86

TG, triglyceride; Prot, protein; HTG, hypertriglyceridemic subject; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; CD, conjugated dienes; Study 1, comparison with primary LPL-deficient subject during pregnancy; Study 2, comparison with primary LPL-deficient subject post pregnancy.

[a]Analysis of 300 μg TG/ml.

[b]Analysis of 60 μg prot/ml.

[c]Assay not performed.

The gel was visualized with Coomassie blue staining. ApoE content was assessed in the same delipidated samples using 12% SDS-PAGE. A portion of this gel was transferred to nitrocellulose for a Western blot analysis using a polyclonal goat anti-human apoE antibody (1:10,000). A second antibody with peroxidase activity was used to catalyze a chemiluminescence detection system (Amersham Life Science, Arlington Heights, IL). The autoradiograph was scanned using a Hewlett-Packard ScanJet IIcx and quantified with Image Quant system (Molecular Dynamics).

Statistical analysis

Statistical analysis was done using *t*-test for paired and unpaired samples when appropriate. A P value of < 0.05 was taken as significant.

RESULTS

Oxidation studies

Measures of oxidation were performed on the TG-rich lipoproteins isolated from the subject with primary LPL deficiency and from the control subjects (**Table 2**). In the study, during her pregnancy, TBARS of TG-rich lipoproteins were only slightly higher in the LPL-deficient subject [3.9 nmol malondialdehyde (MDA)/mg protein] as compared to one of each of the hypertriglyceridemic subjects with normal LPL (3.2 nmol MDA/mg protein) and normolipidemic controls (1.9 nmol MDA/mg protein). However, TBARS values for the remaining hypertriglyceridemic subject and normolipidemic control were higher than the LPL-deficient subject (5.6 and 5.9 nmol MDA/mg protein, respectively). When the TBARS assay was based on 300 μg TG, the values were similar at all time points. Lipid peroxide (LPO) values based on 60 μg protein were generally comparable for the LPL-deficient subject (20.7), hypertriglyceridemic subject 2 (12.3), and normolipidemic control pool (26.5 μmol H_2O_2 equivalents/mg), respectively. When expressed per 300 μg TG, the values were similar. Lag time for conjugated diene formation was longest for the LPL-deficient subject (318 min and propagation rate was lowest (0.32 ΔA_{234}/h), relative to both hypertriglyceridemic and normotriglyceridemic controls. Values for lag time in the hypertriglyceridemic (267 and 103 min) and control (151 and 153 min) subjects were lower. Results were the same when expressed per 60 μg protein. This indicates a greater resistance to oxidation, rather than increased susceptibility as hypothesized. The values post-partum were not markedly different for the LPL-deficient subject as compared to the controls.

Degradation of TG-rich lipoproteins by macrophages

Human monocyte-derived macrophages were utilized to measure uptake and degradation of ^{125}I-labeled TG-rich lipoproteins. Two experiments comparing the degradation of TG-rich lipoproteins from the LPL-deficient subject during pregnancy to two different hypertriglyceridemic subjects and normolipidemic control pools were performed. A different macrophage donor was used for the two experiments. In both instances, the uptake and degradation of the TG-rich lipoproteins from the LPL-deficient subjects was markedly elevated as compared to any of the controls (**Fig. 2**). At a concentration of 50 μg/ml ^{125}I-labeled TG-rich lipoprotein, 927 and 1844 ng TG-rich lipoprotein was degraded/mg cell protein in 5 h in the LPL-deficient study subject in experiments A and B, respectively. This is considerably higher than the values for the two hypertriglyceridemic controls of 429 and 352 ng/mg cell protein in experiments A and B, respectively. The value for the normolipidemic control group in experiment B was even lower, at 126 ng/mg protein. Thus, it appears that the rate of internalization and degradation by macrophages

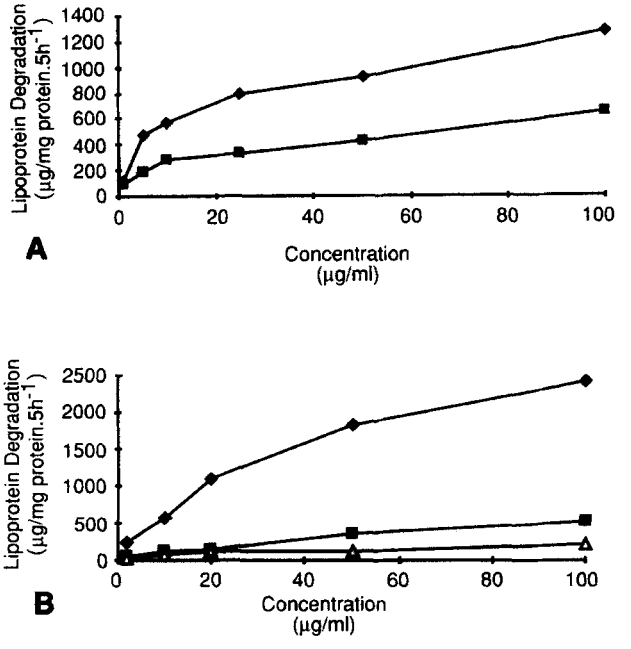

Fig. 2. Degradation of ^{125}I-labeled TG-rich lipoproteins by macrophages from the pregnant LPL-deficient subject. Cells were isolated and plated as described in Methods. Lipoproteins were added to the wells at the concentrations indicated in Experiments 1 and 2, incubated with the cells for 5 h at 37°C, after which medium was removed and lipoprotein degradation products in the incubation medium were assayed as described in Methods. Each data point represents the average of triplicate wells. Experiments 1 and 2 used different macrophage donors. ^{125}I-labeled TG-rich lipoproteins from: pregnant LPL deficient subject (◆), hypertriglyceridemic control-1 (■), Experiment A; pregnant LPL-deficient subject (◆); hypertriglyceridemic control-2 (■), normolipidemic pool-1 (△), Experiment B.

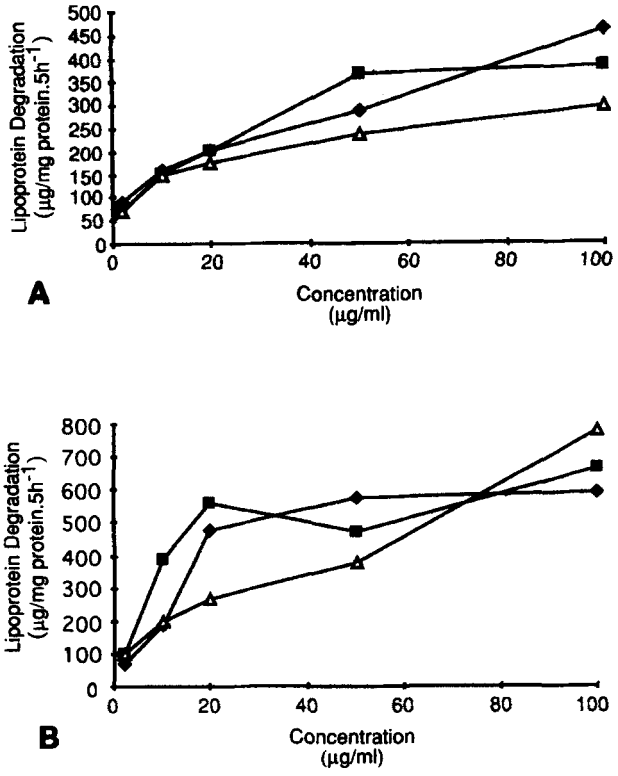

Fig. 3. Degradation of ^{125}I-labeled TG-rich lipoproteins by macrophages from LPL-deficient subject post-pregnancy. Conditions were as described in the legend to Fig. 1. Experiments A and B used similar lipoprotein samples as in the previous experiment, and different macrophage donors. Each data point represents the average of triplicate wells. ^{125}I-labeled TG-rich lipoproteins from: post-pregnant LPL deficient subject (◆), hypertriglyceridemic control-1 (■), normolipidemic pool-2 (△).

is greater for the LPL-deficient particles than either of the controls, given that the degraded counts for the LPL-deficient individual were substantially higher than any of the controls in both experiments.

When TG-rich lipoproteins from the LPL-deficient subject were analyzed 6 months after parturition, at a concentration of 50 μg/ml ^{125}I-labeled TG-rich lipoproteins, values for ^{125}I-labeled lipoprotein degraded were comparable to the hypertriglyceridemic and normolipidemic control pool samples (289, 369, and 240 ng/mg protein per 5 h, respectively) (**Fig. 3**). These values are in the same range as the controls in the previous experiment. The post-pregnancy macrophage degradation experiment was repeated using a different macrophage donor, with similar results in experiments A and B. Therefore, the increased uptake by macrophages of the primary LPL-deficient subject's TG-rich lipoproteins lasted only during pregnancy while the hormonal milieu was altered and the TG-rich lipoproteins were dramatically elevated.

Competition studies (Fig. 4)

Competitors of binding to macrophage lipoprotein receptors were used to elucidate the mechanism of uptake of the TG-rich lipoprotein particles by macrophages. AcLDL represents the control for uptake by the scavenger receptor. The TG-rich lipoproteins from the hypertriglyceridemic controls were used to represent uptake by the LDL receptor or the macrophage specific receptor for TG-rich lipoproteins as described by Gianturco et al. (37). Activated α_2-macroglobulin was added to compete for uptake by the LDL-receptor related protein (LRP). Activated α_2-macroglobulin and TG-rich lipoproteins were used together to determine whether synergism was occurring between these two receptors or whether one was contributing the majority of the effect. The unlabeled competitors were added at 2-, 4-, and 20-fold the concentration of the radiolabeled lipoproteins, which were added to each well at a concentration of 5 μg/ml.

AcLDL competed poorly with the radiolabeled lipoproteins from both the primary LPL-deficient subject and the hypertriglyceridemic control (19% and 5% decrease in ng ^{125}I-labeled lipoproteins degraded/mg cell protein, respectively at 4-fold excess of unlabeled lipoprotein), with a slightly greater effect seen when added to the LPL-deficient subject's radiolabeled lipoproteins. These findings suggest that there is little involvement of the scavenger receptor in the uptake of the TG-rich lipoprotein particles from the hypertriglyceridemic control subject, but may account for some of the uptake seen in the LPL-deficient subject. Activated α_2-macroglobulin had similar effects when added to radiolabeled lipoproteins from the LPL-deficient subject or from hypertriglyceridemic controls (37 to 31% decrease at 2-fold excess concentration in the LPL-deficient and HTG subject, respectively, and 37% decrease in both at 4-fold excess). Therefore, uptake by the LRP does not appear to account for the preferential macrophage uptake of the particles from the LPL-deficient subject. When unlabeled TG-rich lipoprotein particles were added at a 2-fold excess to the ^{125}I-labeled TG-rich lipoproteins from the LPL-deficient subject, decreases in degradation of only 7 and 42% were seen with unlabeled lipoprotein from the LPL-deficient and hypertriglyceridemic control, respectively. Even at a 4-fold excess of unlabeled lipoprotein, the decrease in degradation was only 33 and 49%. When the same unlabeled TG-rich lipoproteins were added to the ^{125}I-labeled TG-rich lipoproteins from a hypertriglyceridemic control subject, the unlabeled ligand competed effectively with the ^{125}I-labeled TG-rich lipoprotein, resulting in large decreases in degradation even at a level of 2-fold excess of unlabeled lipoprotein (73 and 66% decrease with addition of unlabeled LPL-deficient and hyper-

triglyceridemic TG-rich lipoproteins, respectively). This indicates that the TG-rich lipoproteins from the LPL-deficient subject during pregnancy had much greater affinity toward the receptor or uptake mechanism, and therefore were not able to be competed away by the excess unlabeled ligand.

Composition of the TG-rich lipoproteins

Plasma and the TG-rich lipoprotein particles were analyzed for cholesterol and triglyceride content, and levels of apolipoproteins (apo) B and E (**Table 3**). The plasma from the LPL-deficient subject was characterized additionally by DGUC, with cholesterol analysis done on each of the fractions (**Fig. 5**). This revealed the expected pattern in this patient of little to no HDL, LDL, and IDL particles, with the bulk of the lipids carried in a large pool of TG-rich lipoproteins in the range of d < 1.006 g/ml (fractions 30–38). This is in contrast to the profiles for a hypertriglyceridemic control subject (HTG-1) and normolipidemic controls (Fig. 5) where the majority of the cholesterol is found in the range of d 1.019–1.063 (fractions 6–18). The apoB distribution of the TG-rich lipoproteins was examined using SDS-PAGE and revealed both B-48 and B-100 particles present in the "chylomicron" and non-chylomicron "VLDL" fractions (**Fig. 6**). This would indicate a heterogeneous mixture of particles that do not undergo much remodeling or hydrolysis. ApoE distribution was also examined using SDS-PAGE with Western blot analysis and was found to be present in both fractions, but predominantly (approximately 58% greater) in the non-chylomicron fraction (**Fig. 7**). The TG-rich particles isolated for use in this study were d < 1.006 g/ml after a rapid spin to remove chylomicrons. This is the range normally termed VLDL. This TG-rich fraction was used because exogenously derived lipoproteins were a relatively small proportion of the TG-rich lipoproteins in the LPL-deficient subject on a very strict low fat diet. Additionally, even in the postprandial state, 80% of the apoB has been shown to be B-100 of hepatic origin (38).

ApoE isoforms were determined, with the LPL-deficient subject and the HTG-2 subject having a 4/3 phenotype, and the HTG-1 subject being homozygous 3/3. Plasma levels of apoE were elevated in the LPL-deficient subject during pregnancy, (94 mg/dl), a mean value of four determinations over 3 months, and subsequently declined to a mean of 16 mg/dl post-partum. ApoE in the LPL-deficient subject's TG-rich lipoprotein was significantly elevated during pregnancy (168 mg/dl TG-rich lipoprotein) as compared to the hypertriglyceridemic controls (91 and 44 mg/dl) or normolipidemic pooled controls (71 and 45 mg/dl). The apoE level remained elevated even when expressed as the mass ratio of apoE:apoB (0.97 vs. 0.51 and 0.35 for hypertriglyceridemic controls or 0.38 and 0.36 for normolipidemic controls), or as apoE:TG (0.4 vs. 0.7 and 0.2 for hypertriglyceridemic controls or 0.2 and 0.2 for normolipidemic controls). The apoE level fell post-partum to nearly the same level as the hypertriglyceridemic or normolipidemic controls. ApoE levels in the TG-rich lipoproteins from all subjects correlated significantly with their respective uptake and degradation of the lipoproteins by macrophages ($r = 0.84$; $P < 0.01$). This positive relationship is consistent with apoE mediating the increased uptake and degradation of the TG-rich lipoproteins (**Fig. 8**).

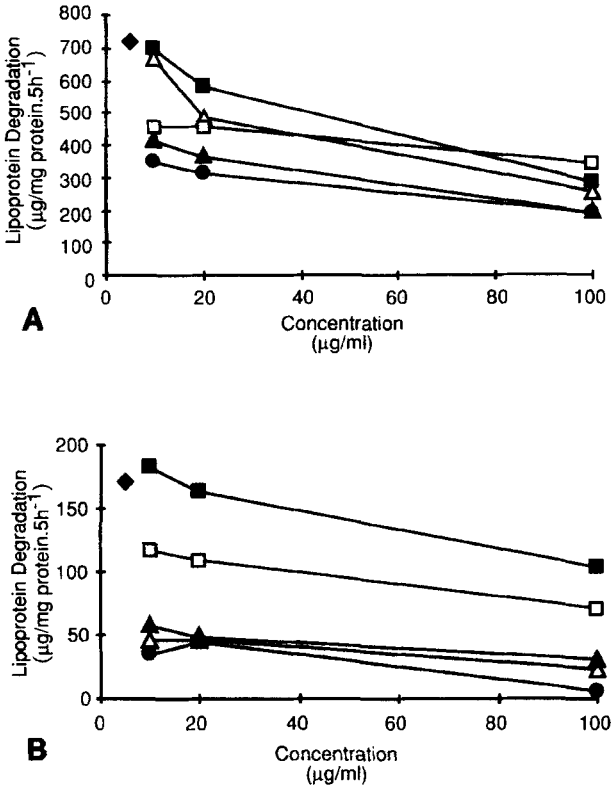

Fig. 4. Competitive uptake and degradation of TG-rich lipoproteins by macrophages. Cells were isolated and plated as described in methods. ^{125}I-labeled TG-rich lipoproteins (5 μg/ml) were added to the cells along with unlabeled competitors added at 2-, 4-, or 20-fold excess concentration as compared to the radiolabeled lipoprotein. Conditions were as described in the legend to Fig. 1. Experiment 4A used a baseline concentration of 5 μg/ml ^{125}I-labeled TG-rich lipoprotein from the pregnant LPL-deficient subject (◆). Experiment 4B used a baseline concentration of 5 μg/ml ^{125}I-labeled TG-rich lipoprotein from subject hypertriglyceridemic control-2 (◆). Each data point represents the average of triplicate wells. Unlabeled competitors: AcLDL (■), pregnant LPL-deficient subject TG-rich lipoprotein (△), hypertriglyceridemic-2 TG-rich lipoprotein (▲), activated α_2-macroglobulin (□), activated α_2-macroglobulin + hypertriglyceridemic-2 TG-rich lipoprotein in Experiment A and activated α_2-macroglobulin + pregnant LPL-deficient TG-rich lipoprotein in

TABLE 3. Compositional data: TG-rich lipoproteins

Subject	ApoE:ApoB	ApoE:TG	ApoE Isoform
LPL-deficient (pregnant)	0.97	0.04	4/3
LPL-deficient (post-pregnancy)	0.41	0.03	
HTG-1 (Study 1)	0.26	0.01	3/3
HTG-1 (Study 2)	0.51	0.07	
HTG-2 (Study 1)	0.35	0.02	4/3
Normolipidemic pool (Study 1)	0.38	0.02	[a]
Normolipidemic pool (Study 2	0.36	0.02	[a]

TG, triglycerides; Apo, apolipoprotein; Study 1, comparison with primary LPL-deficient subject during pregnancy; Study 2, comparison with primary LPL-deficient subject post pregnancy.
[a]Not performed because pooled plasma was used.

DISCUSSION

The appearances of palmar xanthomata in a pregnant subject with primary LPL deficiency is unusual, as palmar xanthomata usually occur when remnants of the TG-rich lipoproteins (TG-rich lipoproteins from which TGs have been partially hydrolyzed by LPL) accumulate in plasma. It is unclear whether this situation is unique to this subject as there is limited information regarding LPL deficiency in pregnancy in general. However, it has not been described in the literature prior to this report. As palmar xanthomata are comprised of lipid-laden macrophages, we evaluated the uptake and degradation of this subject's TG-rich lipoproteins by macrophages in vitro.

We demonstrated that uptake and degradation of TG-rich lipoproteins from the pregnant LPL-deficient subject by monocyte-derived macrophages was considerably greater than was the uptake and degradation of TG-rich lipoproteins from hypertriglyceridemic subjects with normal LPL and from normolipemic controls. Macrophages secrete LPL which can act as a ligand to facilitate binding and uptake of lipoproteins (39). Had the LPL-deficient subject's macrophages been used, which do not secrete active LPL, a blunting of the results may have been seen. However, the magnitude of the increased uptake and degradation of the TG-rich lipoproteins in this subject was clinically significant, in that she developed palmar xanthoma, which are due to deposition of lipid in macrophages. This could potentially be attributed to increased oxidative modification of her TG-rich lipoproteins with subsequent uptake by macrophage receptors, or to their increased uptake by macrophages due to other factors, such as an increased level of apoE. Analyses of indices of lipid peroxidation in freshly isolated TG-rich lipoproteins and their susceptibility to Cu-mediated oxidation ex vivo did not indicate large differences between the subjects, with the exception that the TBARS and LPO values were slightly higher in the pregnant LPL-deficient subject. Taken together, these data do not support the hypothesis that increased oxidative modification of the lipoproteins was the main factor contributing to the increased macrophage uptake and formation of palmar xanthoma in this pregnant subject with primary LPL deficiency.

Uptake of TG-rich lipoproteins by macrophages can occur via several mechanisms. Uptake of TG-rich lipoproteins by the LDL receptor on macrophages and other cell types is mediated by apoE (40, 41). TG-rich lipoproteins from some hypertriglyceridemic individuals have a higher affinity for the LDL receptor than do particles from normotriglyceridemic individuals (42). Gianturco et al. (43) and Ramprasad et al. (44) have also shown that there is a distinct macrophage receptor for chylomicrons and hypertriglyceridemic VLDL. ApoE is not a ligand for this receptor. Scavenger receptors are present on macrophages and can bind TG-rich lipopro-

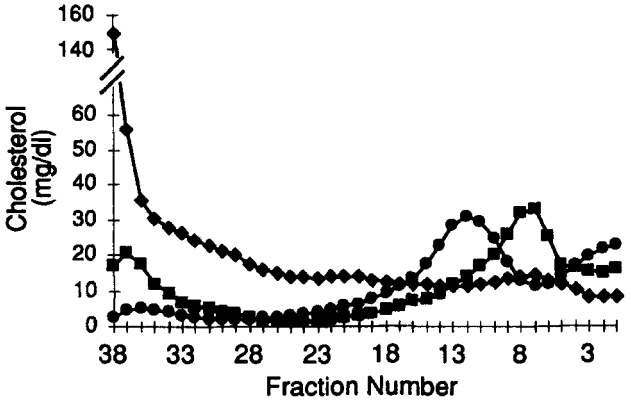

Fig. 5. Lipoprotein distribution in LPL-deficient and control subjects. Plasma (1 ml) was subjected to density gradient ultracentrifugation as described in Methods. Thirty eight fractions were collected and assayed for cholesterol. High density lipoprotein (HDL, d 1.063–1.125 g/ml) is in fractions 1–5; low density lipoprotein (LDL, d 1.019–1.063 g/ml) is in fractions 6–18; intermediate density lipoprotein (IDL, d 1.006–1.019 g/ml) is in fractions 18–30; and very low density lipoprotein (VLDL, d 1.006 g/ml) is in fractions 30–38. LPL-deficient subject (◆), hypertriglyceridemic control-1 (■), normolipidemic pooled controls (●). Data for the normolipidemic subjects are from reference 35.

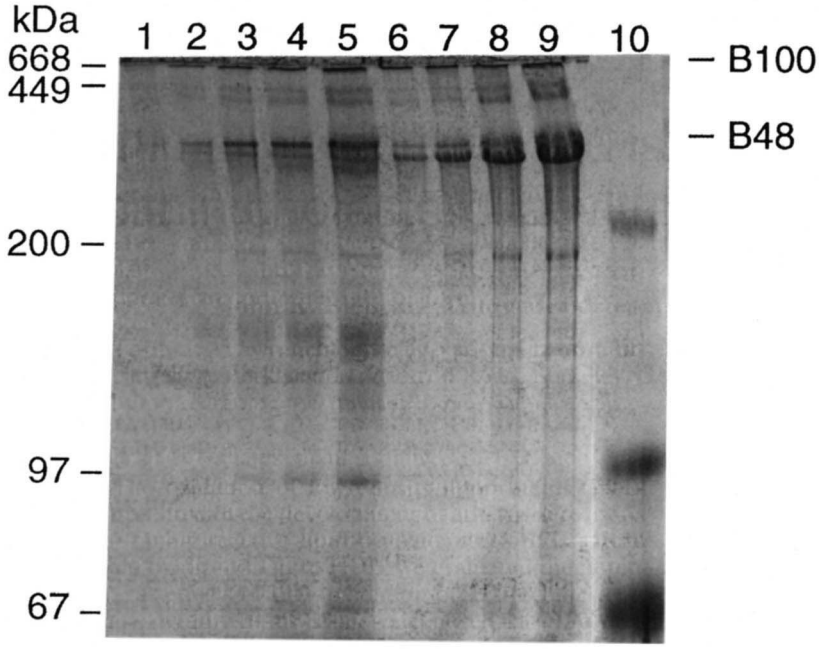

Fig. 6. SDS-PAGE for apoB. Delipidated TG-rich lipoproteins were electrophoresed in a 5% SDS-PAGE as described in Methods. Chylomicrons (lanes 2–5); non-chylomicron TG-rich lipoproteins (lanes 6–9); standards (lanes 1 and 10). Volumes loaded in lanes 2 and 6, 5 μl; 3 and 7, 10 μl; 4 and 8, 25 μl; 5 and 9, 50 μl. ApoB-100 molecular mass = 512.7 kDa; apoB-48 molecular mass = 240.8 kDa.

teins that have become sufficiently oxidized such that they are recognized by this family of receptors (45). Other receptors involved include the LRP, which binds apoE-rich LP particles as well as α_2-macroglobulin/protease complexes, and the receptor-associated protein which is thought to regulate binding to this receptor (46). The VLDL receptor has been identified recently in rabbit and human tissues; however, it does not appear to be expressed to a large extent in macrophages (47, 48). Therefore, this receptor is unlikely to be relevant to the present study.

Compositional analysis showed a significant elevation in the apoE content of the LPL-deficient subject's TG-rich lipoproteins only during pregnancy. ApoE remained elevated even when adjusted per particle (apoB) and for lipid level. Estrogen has been shown to stimulate the production of large VLDL particles (49), accounting for the physiologic rise in TG seen during the second and third trimesters. Production of apoB also is stimulated by estrogen in premenopausal women (50). Conversely, plasma apoE levels have been reported to decrease slightly with estrogen treatment (51). However, in that study, it is of interest that the distribution of the apolipoprotein was altered, with a 46% increase in VLDL apoE content, not adjusted for apoB (51). ApoE metabolism during pregnancy has been investigated in a mouse model. A 10- and 5-fold increase in apoE mRNA levels was observed in placenta and uterus, respectively (52). The dramatic increase in plasma apoE levels in the LPL-deficient subject may reflect the large increase in placental and uterine apoE that might not be observed in individuals who can adequately clear the increased VLDL levels. There also is the possibility that the LPL-deficient subject may have had a higher proportion of apoE-enriched TG-rich lipoproteins than normal individuals prior to pregnancy. This is suggested by a report of Campos et al. (53) where a monoclonal antibody to apoB-100 that does not recognize apoE-rich B-100 particles or B-48 particles was used to separate populations of TG-rich lipoproteins. They found that 65% of TG-rich lipoproteins from a LPL-deficient subject were relatively enriched in apoE as compared to 10–13% of TG-rich lipoproteins from normotriglyceridemics and 10–29% from hypertriglyceridemic individuals. It is also conceivable that the very long residence time of the TG-rich lipoproteins, consequent to her LPL deficiency and pregnancy, exacerbated her hypertriglyceridemia, and resulted in redistribution of apoE, apoC-II, and apoC-III. Thus, the normal lipid and lipoprotein changes associated with the hormonal milieu of pregnancy appear to have been markedly exacerbated by the subject's primary LPL deficiency, resulting in severe hypertriglyceridemia, as has been previously described, and enrichment of TG-rich lipoproteins with apoE (54).

In addition to the profound effects of estrogen on VLDL production and apoE expression, it has been known for some time that the hormone inhibits hepatic lipase (HL) activity (55). This inhibition can result in a 75% reduction in postheparin plasma HL activity during the second and third trimesters of pregnancy (56). HL facilitates hepatic uptake and endocytosis of chylomicron remnants and VLDL (57). The LPL-deficient subject may have developed a greater physiologic reliance on HL for removal of her TG-rich lipoproteins. When

this enzyme was inhibited during pregnancy, the subject was missing both mediators of TG-rich lipoprotein remodeling and removal, further exacerbating the physiologic hypertriglyceridemia of pregnancy. Therefore, the cumulative effect of absolute LPL deficiency and the relative HL deficiency due to pregnancy, as well as increased TG-rich lipoprotein production and apoE enrichment induced by estrogen, may have resulted in a setting where macrophage lipid uptake was facilitated.

The increased uptake of this subject's TG-rich lipoproteins by macrophages was likely to have been apoE-dependent, as this uptake and degradation returned towards normal post-pregnancy concomitant with a reduction in the apoE content of her TG-rich lipoproteins. Further, a significant correlation between the apoE content of TG-rich lipoproteins and degradation by macrophages was observed. VLDL are a very heterogeneous population of particles, particularly in hypertriglyceridemic subjects, as the proportion of large particles increases with increasing plasma TG. The DGUC profile, as well as distribution of apoE, and apoB-100 and B-48 between the "chylomicron" fraction and the TG-rich "VLDL" fraction supports the premise that the TG-rich lipoproteins in the LPL-deficient subject were a heterogeneous mixture of very large particles that did not undergo much remodeling or hydrolysis. Previous studies with emulsions and native lipoproteins have shown that exogenous apoE enhances the uptake of particles by macrophages, and that the effect is greatest with large particles (58, 59). The LDL receptor has multiple ligand binding sites and the amount of apoE on the lipoprotein particle increases its binding affinity to the receptor (60, 61). Our compositional data indicating a high enrichment of particles from the LPL-deficient pregnant subject with apoE is consistent with a model whereby a large, apoE-rich TG-rich lipoprotein has a higher affinity for the LDL receptor than that from any of the other subjects tested. Particle size and number of apo-E molecules also are thought to be factors that determine the pattern of endocytotic targeting. Large, apoE-rich β-VLDL were found to be targeted to widely distributed vesicles and had a higher acyl-CoA cholesterol acyltransferase (ACAT) stimulatory potential than smaller, or less apoE-rich particles (62). This may help explain the unusual uptake and accumulation of lipid in the macrophages in the study subject that resulted in the appearance of the palmar xanthoma. Additionally, as hepatic LDL receptor activity is increased with estrogen treatment in rodents and humans (63, 64), it is possible that macrophage LDL receptors are similarly up-regulated. As the macrophage receptor for TG-rich lipoproteins described by Gianturco et al. (43) does not bind apoE, it is unlikely that this receptor accounted for the increased uptake of TG-rich lipoproteins observed.

The competition studies shed some light on the mechanism(s) that may be contributing to the uptake of lipid by the macrophages and subsequent appearance of the xanthoma in the LPL-deficient subject. The greatest competition for degradation of both the LPL-deficient and hypertriglyceridemic radiolabeled TG-rich lipoprotein was by the LPL-deficient subject's unlabeled lipoprotein, consistent with uptake by an apoE-dependent mechanism. Competition with activated α_2-macroglobulin suggested that there is not preferential uptake

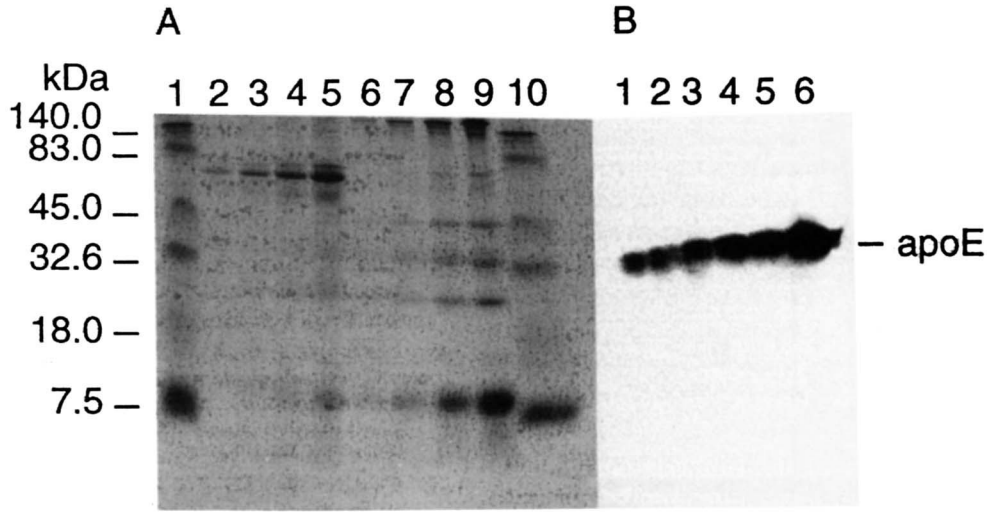

Fig. 7. SDS-PAGE for apoE. Delipidated samples as described in the legend to Fig. 6 were electrophoresed by 12% SDS-PAGE. (A) Coomassie stain. Lanes and samples are as described in Fig. 6. ApoE molecular mass = 34.2 kDa. (B) Western blot autoradiograph of chemiluminescence detection. Chylomicrons (lanes 1–3), non-chylomicron TG-rich lipoproteins (lanes 4–6). Volumes loaded in lanes 1 and 4, 5 μl; lanes 2 and 5, 10 μl; lanes 3 and 6, 25 μl.

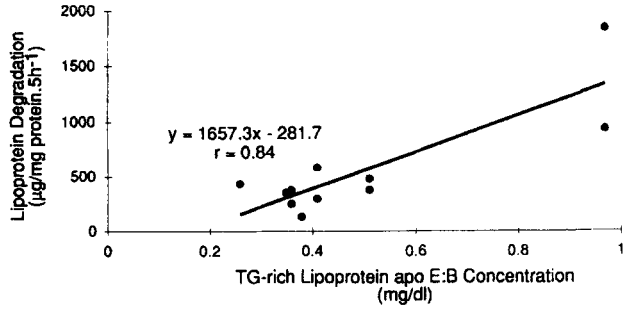

Fig. 8. Relationship between TG-rich lipoprotein apoE:B concentration from all subjects and respective degradation of ^{125}I-labeled TG-rich lipoprotein by macrophages.

of the TG-rich lipoproteins from the LPL-deficient subject by the LRP, and that this receptor is not responsible for the uptake of the bulk of the TG-rich lipoproteins. However, the current model of ligand binding to LRP is that the activated α_2-macroglobulin and apoE-enriched lipoproteins bind to distinct sites on the receptor and that the partial inhibition seen in this and other studies is due to steric hindrance between the two ligand molecules (65). Thus, the LRP may still represent a compensatory mechanism to partially explain the uptake of TG-rich LP in the LPL-deficient subject during pregnancy due to the excessively high levels of TG and the unusual enrichment of the particles with apoE. However, the data indicate that the macrophage LDL (apoB/E) receptor was the primary receptor with which the apoE-enriched TG-rich lipoproteins were interacting.

In summary, the increased uptake and degradation of the TG-rich lipoproteins from the LPL-deficient subject by macrophages appear to be related to the unusually high apoE content that was present during pregnancy, and was resolved post-partum. Based on the competition data, the affinity of the lipoprotein particles from the LPL-deficient subject for the macrophage receptors appeared to be greater than in control subjects. This is consistent with other experimental data showing increased affinity of large, apoE-enriched TG-rich lipoprotein particles for the LDL receptor, and differential intracellular targeting of these particles. Thus, compositional and functional changes could potentially contribute to the unusual palmar xanthoma observed in this woman with primary LPL deficiency.

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REFERENCES

1. Brunzell, J. D. 1995. Lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. *In* The Metabolism and Molecular Bases of Inherited Disease, 7th edition. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1913–1932.
2. Hayden, M. R., J. J. Kastelein, H. Funke, J. D. Brunzell, and Y. Ma. 1993. Phenotypic variation of mutations in the human lipoprotein lipase gene. *Biochem. Soc. Trans.* **21:** 506–509.
3. Lykkesfeldt, G., J. E. Bock, F. D. Pederson, H. Meinertz, and O. Faergeman. 1980. Excessive hypertriglyceridemia and pancreatitis in pregnancy. *Acta Obstet. Gynecol. Scand.* **60:** 79–82.
4. Steiner, G., J. J. Myher, and A. Kuksis. 1995. Milk and plasma lipid composition in a lactating patient with type I hyperlipoproteinemia. *Am. J. Clin. Nutr.* **41:** 121–128.
5. DeChalain, T. M. B., W. L. Michell, and G. M. B. Berger. 1988. Hyperlipidemia, pregnancy and pancreatitis. *Surg. Gynecol. Obstet.* **167:** 469–473.
6. Watts, G. F., K. Morton, P. Jackson, and B. Lewis. 1992. Management of patients with severe hypertriglyceridemia during pregnancy: report of two cases with familial lipoprotein lipase deficiency. *Br. J. Obstet. Gynaecol.* **99:** 163–166.
7. Ma, Y., M-S. Liu, D. Ginzinger, J. Frohlich, J. D. Brunzell, and M. R. Hayden. 1993. Gene-environment interaction in the conversion of a mild to severe phenotype in a patient homozygous for a Ser^{172}-Cys mutation in the lipoprotein lipase gene. *J. Clin. Invest.* **91:** 1953–1958.
8. Nies, B. M., and R. J. Dreiss. 1990. Hyperlipidemic pancreatitis in pregnancy: a case report and review of the literature. *Am. J. Perinatol.* **7:** 166–169.
9. Mahley, R. W. 1991. Type III hyperlipoproteinemia (dysbetalipoproteinemia): a genetic disorder associated with premature atherosclerosis. *In* Primary Hyperlipoproteinemias. G. Steiner and G. Shafrir, editors. McGraw-Hill, New York. 261–269.
10. Lewis, B. 1976. The combined hyperlipidaemias. *In* The Hyperlipidaemias—Clinical and Laboratory Practice. Blackwell Scientific Publications, Oxford, UK. 276–291.
11. Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown. 1979. Binding site on macrophages that mediate uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA.* **76:** 333–337.
12. Potter, J. M., and P. J. Nestel. 1979. The hyperlipidemia of pregnancy in normal and complicated pregnancies. *Am. J. Obstet. Gynecol.* **133:** 165–170.
13. Knopp, R. H., R. O. Bergelin, P. W. Wahl, C. E. Walden, M. Chapman, and S. Irvine. 1982. Population-based lipoprotein lipid reference values for pregnant women compared to nonpregnant women classified by sex hormone usage. *Am. J. Obstet. Gynecol.* **143:** 626–637.

14. Warth, M. R., R. A. Arky, and R. H. Knopp. 1975. Lipid metabolism in pregnancy. III. Altered lipid composition in intermediate, very low, low and high density lipoprotein fractions. *J. Clin. Endocrinol. Metab.* **41:** 649–655.
15. Herrera, E., M. A. Lusuncion, D. Gomez-Coronada, P. Arando, P. Lopez-Luna, and I. Maier. 1988. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am. J. Obstet. Gynecol.* **158:** 1575–1583.
16. Brunzell, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
17. Havel, R. J., and J. P. Kane. 1973. Primary dysbetalipoproteinemia: predominancy of a specific apoprotein species in triglyceride-rich lipoproteins. *Proc. Natl. Acad. Sci. USA.* **70:** 2015–2019.
18. Krul, E. S., M. J. Tikkanen, T. G. Cole, J. M. Davie, and G. Schonfeld. 1985. Roles of apolipoproteins B and E in the cellular binding of very low density lipoproteins. *J. Clin. Invest.* **75:** 361–369.
19. Iverius, P-H., and J. D. Brunzell. 1985. Human adipose tissue lipoprotein lipase: changes with feeding and relation to postheparin plasma enzyme. *Am. J. Physiol.* **249:** E107–E114.
20. Press, M., P. J. Hartrop, and C. Prottey. 1974. Correction of essential fatty-acid deficiency in man by the cutaneous application of sunflower-seed oil. *Lancet.* **1:** 597–598.
21. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. *Biochim. Biophys. Acta.* **260:** 212–221.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
23. Chung, B. H., T. Wilkinson, J. C. Geer, and J. P. Segrest. 1980. Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. *J. Lipid Res.* **21:** 284–291.
24. Basu, S. K., J. L. Goldstein, R. G. W. Anderson, and M. S. Brown. 1976. Degradation of catio˜ized low density lipoproteins and regulation of cholesterol metabolism in homozygous familial hypercholesterolemia fibroblasts. *Proc. Natl. Acad. Sci. USA.* **73:** 3178–3182.
25. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Invest.* **21:** 77–99.
26. VanLeuven, F., J. J. Cassiman, and X. van den Berghe. 1981. Functional modifications of α_2-macroglobulin by primary amines. I. Characterization of α2M after derivatization by methylamine and by Factor XIII. *J. Biol. Chem.* **256:** 9016–9022.
27. Bierman, E. L., O. Stein, and Y. Stein. 1974. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. *Circ. Res.* **35:** 136–150.
28. Buege, J. A., and S. D. Aust. 1978. Microsomal lipid peroxidation. *Methods Enzymol.* **52:** 302–310.
29. El-Saadani, M., H. Esterbauer, M. El-Sayed, M. Goher, A. Y. Nassar, and G. Jurgens. 1989. A spectrophotometric assay for lipid peroxides in serum lipoprotein using a commercially available reagent. *J. Lipid Res.* **30:** 627–630.
30. Esterbauer, H., G. Striegl, H. Puhl, and M. Rotheneder. 1989. Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radic. Res. Commun.* **6:** 67–75.
31. Tsai, E. C., I. B. Hirsch, J. D. Brunzell, and A. Chait. 1994. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes.* **43:** 1010–1014.
32. Marcovina, S. M., J. J. Albers, H. Kennedy, J. V. Mei, L. O. Henderson, and W. H. Hannon. 1994. International Federation of Clinical Chemistry Standardization Project for measurements of apolipoproteins A-I and B. IV. Comparability of apolipoprotein B values by use of International Reference Material. *Clin. Chem.* **40:** 586–592.
33. Applebaum-Bowden, D. M., P. McLean, A. Steinmetz, D. Fontana, C. Matthys, G. R. Warnick, M. Cheung, J. J. Albers, and W. R. Hazzard. 1989. Lipoprotein, apolipoprotein, and lipolytic enzyme changes following estrogen administration in postmenopausal women. *J. Lipid Res.* **30:** 1895–1906.
34. Kataoka, S., M. Paidi, and B. V. Howard. 1994. Simplified isoelectric focusing/immunoblotting determination of apoprotein E phenotype. *Clin. Chem.* **40:** 11–13.
35. Hokanson, J. E., M. A. Austin, A. Zambon, and J. D. Brunzell. 1993. Plasma triglyceride and LDL heterogeneity in familial combined hyperlipidemia. *Arterioscler. Thromb.* **13:** 427–434.
36. Laemmli, U. K. 1970. Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
37. Gianturco, S. H., W. A. Bradley, A. M. Gotto, Jr., J. D. Morrisett, and D. L. Peavy. 1982. Hypertriglyceridemic very low density lipoproteins induce triglyceride synthesis and accumulation in mouse peritoneal macrophages. *J. Clin. Invest.* **70:** 168–178.
38. Schneeman, B. O., L. Kotite, K. M. Todd, and R. J. Havel. 1993. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc. Natl. Acad. Sci. USA.* **90:** 2069–2073.
39. Takahashi, S., J. Suzuki, M. Kohno, K. Oida, T. Tamai, S. Miyabo, T. Yamamoto, and T. Nakai. 1995. Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase. *J. Biol. Chem.* **270:** 15747–15754.
40. Huff, M. W., A. J. Evans, C. G. Sawyez, B. M. Wolfe, and P. J. Nestel. 1991. Cholesterol accumulation in J744 macrophages induced by triglyceride-rich lipoproteins. Comparison of very low density lipoprotein from subjects with type III, IV and V hyperlipoproteinemias. *Arterioscler. Thromb.* **11:** 221–233.
41. Gianturco, S. H., A. M. Gotto, Jr., S. L. Hwang, J. B. Karlin, A. H. Lin, S. C. Prasad, and W. A. Bradley. 1983. Apolipoprotein E mediates uptake of S_f 100–400 hypertriglyceridemic very low density lipoproteins by the low density lipoprotein receptor pathway in normal human fibroblasts. *J. Biol. Chem.* **258:** 4526–4533.
42. Innerarity, T. L., and R. W. Mahley. 1978. Enhanced binding by cultured human fibroblasts of apoE-containing lipoproteins as compared with low density lipoproteins. *Biochemistry.* **17:** 1440–1447.
43. Gianturco, S. H., M. P. Ramprasad, A. H-Y. Lin, R. Song, and W. A. Bradley. 1994. Cellular binding site and membrane binding proteins for triglyceride-rich lipoproteins

in human monocyte-macrophages and THP-1 monocytic cells. *J. Lipid Res.* **35:** 1674–1687.

44. Ramprasad, M. P., R. Li, S. H. Gianturco, and W. A. Bradley. 1995. Purification of the human THP-1 monocyte-macrophage triglyceride-rich lipoprotein receptor. *Biochem. Biophys. Res. Commun.* **210:** 491–497.
45. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
46. Moestrup, S. K., and J. Gliemann. 1991. Analysis of ligand recognition by the purified α2-macroglobulin receptor (low density lipoprotein receptor-related protein). *J. Biol. Chem.* **266:** 14011–14017.
47. Gåfvels, M. E., M. Caird, D. Britt, C. L. Jackson, D. Patterson, and J. F. Strauss. 1993. Cloning of a cDNA encoding a putative human very low density lipoprotein/apolipoprotein E receptor and assignment of the gene to chromosome 9pter-p236. *Somat. Cell. Mol. Genet.* **19:** 557–564.
48. Takahashi, S., Y. Kawarabayasi, N. Tsuguhiko, J. Sakai, and T. Yamamoto. 1992. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. USA.* **89:** 9252–9256.
49. Walsh, B. W., and F. M. Sacks. 1993. Effects of low dose oral contraceptives on very low density and low density lipoprotein metabolism. *J. Clin. Invest.* **91:** 2126–2132.
50. Schaefer, E. J., D. M. Foster, L. A. Zech, F. T. Lindgren, H. B. Brewer, Jr., and R. I. Levy. 1983. The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. *J. Clin. Endocrinol. Metab.* **57:** 262–267.
51. Meusing, R. A., V. T. Miller, J. C. LaRosa, D. B. Stoy, and E. A. Phillips. 1992. Effects of unopposed conjugated equine estrogen on lipoprotein composition and apolipoprotein-E distribution. *J. Clin. Endocrinol. Metab.* **75:** 1250–1254.
52. Overbergh, L., K. Lorent, S. Torrekens, F. Van Leuven, and H. Van den Berghe. 1995. Expression of mouse alpha-macroglobulins, lipoprotein receptor-related protein, LDL receptor, apolipoprotein E, and lipoprotein lipase in pregnancy. *J. Lipid Res.* **36:** 1774–1786.
53. Campos, E., K. Nakajima, A. Tanaka, and R. J. Havel. 1992. Properties of an apolipoprotein E-enriched fraction of triglyceride-rich lipoproteins isolated from human blood plasma with a monoclonal antibody to apolipoprotein B-100. *J. Lipid Res.* **33:** 369–380.
54. Salameh, W. A., and D. S. Mastrogiannis. 1994. Maternal hyperlipidemia in pregnancy. *Clin. Obstet. Gyn.* **37:** 66–77.
55. Tikkanen, M. J., and E. A. Nikkilä. 1987. Regulation of hepatic lipase and serum lipoproteins by sex steroids. *Am. Heart J.* **113:** 562–567.
56. Kroon, A. A., D. W. Swinkels, P. W. J. van Dongen, and A. F. H. Stalenhoef. 1994. Pregnancy in a patient with homozygous familial hypercholesterolemia treated with long-term low-density lipoprotein apheresis. *Metabolism.* **43:** 1164–1170.
57. Shafi, S., S. E. Brady, A. Bensadoun, and R. J. Havel. 1994. Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J. Lipid Res.* **35:** 709–720.
58. Granot, E., B. Schwiegelshohn, I. Tabas, M. Gorecki, T. Vogel, Y. A. Carpentier, and R. J. Deckelbaum. 1994. Effects of particle size on cell uptake of model triglyceride rich particles with and without apolipoprotein E. *Biochemistry.* **33:** 15190–15197.
59. Granot, E., and S. Eisenberg. 1990. ApoE-3-specific metabolism of human plasma very low density lipoproteins in cultured J-774 macrophages. *J. Lipid Res.* **31:** 2133–2139.
60. Innerarity, T. L., E. S. Kempner, D. Y. Hui, and R. W. Mahley. 1981. Functional unit of the low density lipoprotein receptor of fibroblasts: a 100,000-dalton structure with multiple binding sites. *Proc. Natl. Acad. Sci. USA.* **78:** 4378–4382.
61. Funahashi, T., S. Yokoyama, and A. Yamamoto. 1989. Association of apolipoprotein E with the low density lipoprotein receptor: demonstration of its co-operativity on lipid microemulsion particles. *J. Biochem.* **105:** 582–587.
62. Tabas, I., J. N. Myers, T. L. Innerarity, X-X. Xu, K. Arnold, J. Boyles, and F. R. Maxfield. 1991. The influence of particle size and multiple apoprotein E-receptor interactions on the endocytotic targeting of β-VLDL in mouse peritoneal macrophages. *J. Cell Biol.* **115:** 1547–1560.
63. Windler, E. E. T., P. T. Kovanen, Y-S. Chao, M. Brown, R. J. Havel, and J. L. Goldstein. 1980. The estradiol-stimulated lipoprotein receptor of rat liver. A binding site that mediates the uptake of rate lipoproteins containing apoproteins B and E. *J. Biol. Chem.* **255:** 10464–10471.
64. Angelin, B., H. Olivecrona, E. Reihner, M. Rudling, D. Ståhlberg, M. Eriksson, S. Ewerth, P. Henriksson, and K. Einarsson. 1992. Hepatic cholesterol metabolism in estrogen-treated men. *Gastroenterology.* **103:** 1657–1663.
65. Hussain, M. M., F. R. Maxfield, J. Mas-Oliva, I. Tabas, Z. S. Ji, T. L. Innerarity, and R. W. Mahley. 1991. Clearance of chylomicron remnants by the low density lipoprotein receptor-related protein/α2-macroglobulin receptor. *J. Biol. Chem.* **266:** 13936–13940.