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
TABLE 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Plasma Chol</th>
<th>Plasma TG</th>
<th>Plasma ApoE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL-deficient (pregnant) female</td>
<td>female</td>
<td>29</td>
<td>284</td>
<td>4518</td>
<td>94</td>
</tr>
<tr>
<td>LPL-deficient (post-pregnancy) female</td>
<td>female</td>
<td>29</td>
<td>122</td>
<td>511</td>
<td>16</td>
</tr>
<tr>
<td>HTG-1 (Study 1) male</td>
<td>male</td>
<td>48</td>
<td>252</td>
<td>728</td>
<td>13</td>
</tr>
<tr>
<td>HTG-1 (Study 2) male</td>
<td>male</td>
<td>48</td>
<td>232</td>
<td>555</td>
<td>*</td>
</tr>
<tr>
<td>HTG-2 (Study 1) female</td>
<td>female</td>
<td>61</td>
<td>240</td>
<td>745</td>
<td>*</td>
</tr>
<tr>
<td>Normolipidemic pool (Study 1) males</td>
<td>males</td>
<td>27-58</td>
<td>190</td>
<td>108</td>
<td>8</td>
</tr>
<tr>
<td>Normolipidemic pool (Study 2) males</td>
<td>males</td>
<td>27-58</td>
<td>167</td>
<td>103</td>
<td>4</td>
</tr>
</tbody>
</table>

Chol, cholesterol; TG, triglyceride; Study 1, comparison with primary LPL-deficient subject during pregnancy; Study 2, comparison with primary LPL-deficient subject post pregnancy.

*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 hyper-triglyceridemic 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
mg/day) as a potentially preventive measure to provide her with a source of essential fatty acids (20). Fenofibrate (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 fenofibrate 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 postpartum.

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 VTi50.2 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 x 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 α2-macroglobulin were added to some of the experiments. Activated α2-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 Cu²⁺-induced oxidation was assessed by monitoring conjugated diene formation by the method of Esterbauer et al. (30). Ten μM CuSO₄ 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 A₂₃₄ 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

<table>
<thead>
<tr>
<th>Subject</th>
<th>TBARS</th>
<th>LPO</th>
<th>CD Lag Time</th>
<th>CD Propagation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG⁴</td>
<td>Prot</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n mol MDA/mg</td>
<td>μmol H₂O₂ equivalents/mg</td>
<td>min</td>
<td>A A₂₃₄/h</td>
</tr>
<tr>
<td>LPL-deficient (pregnant)</td>
<td>0.59</td>
<td>3.9</td>
<td>18.3</td>
<td>20.7</td>
</tr>
<tr>
<td>LPL-deficient (post-pregnancy)</td>
<td>0.77</td>
<td>2.8</td>
<td>27.0</td>
<td>39.8</td>
</tr>
<tr>
<td>HTG-1 (Study 1)</td>
<td>0.62</td>
<td>3.2</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>HTG-1 (Study 2)</td>
<td>0.85</td>
<td>4.2</td>
<td>16.3</td>
<td>5.2</td>
</tr>
<tr>
<td>HTG-2 (Study 1)</td>
<td>1.23</td>
<td>5.6</td>
<td>38.0</td>
<td>12.3</td>
</tr>
<tr>
<td>Normolipidemic pool (Study 1)</td>
<td>1.0</td>
<td>1.9</td>
<td>41.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Normolipidemic pool (Study 2)</td>
<td>0.78</td>
<td>5.9</td>
<td>23.0</td>
<td>26.5</td>
</tr>
</tbody>
</table>

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.

'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 H₂O₂ 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 ΔA₂₃₄₅/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 ¹²⁵I-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/ml¹²⁵I-labeled TG-rich lipoprotein, 927 and 1844 ng TG-rich lipoprotein was degraded/mg cell protein in 5 h in the LPL-deficient subject 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. 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 Gian-turco et al. (37). Activated $\alpha_2$-macroglobulin was added to compete for uptake by the LDL-receptor related protein (LRP). Activated $\alpha_2$-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 $\alpha_2$-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 controls (37 to 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 B48 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).
TABLE 3. Compositional data: TG-rich lipoproteins

<table>
<thead>
<tr>
<th>Subject</th>
<th>ApoE</th>
<th>ApoB</th>
<th>ApoE:TG</th>
<th>ApoE Isoform</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL-deficient (pregnant)</td>
<td>0.97</td>
<td>0.04</td>
<td>4/3</td>
<td></td>
</tr>
<tr>
<td>LPL-deficient (post-pregnancy)</td>
<td>0.41</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTG-1 (Study 1)</td>
<td>0.26</td>
<td>0.01</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>HTG-1 (Study 2)</td>
<td>0.51</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTG-2 (Study 1)</td>
<td>0.35</td>
<td>0.02</td>
<td>4/3</td>
<td></td>
</tr>
<tr>
<td>Normolipidemic pool (Study 1)</td>
<td>0.38</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normolipidemic pool (Study 2)</td>
<td>0.36</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TG, triglycerides; Apo, apolipoprotein; Study 1, comparison with primary LPL-deficient subject during pregnancy; Study 2, comparison with primary LPL-deficient subject post pregnancy.

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 normolipidemic 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-
proteins 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 α2-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 TC-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 mechanisms 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 α2-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 1–5), 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.](image-url)
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 α2-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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