

**Abstract**

Little is known about the immediate effects of dietary fat on the expression of genes involved in cholesterol metabolism in humans. We investigated the effects of a high-fat meal on circulating mononuclear cell messenger RNA (mRNA) for the LDL receptor (LDLR), LDLR-related protein (LRP), and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) over 10 h. Selection of 12 C and 7 T homozygotes for the LRP exon 22 C200T polymorphism for the study also enabled us to examine the influence of this polymorphism on postprandial mRNA expression and lipoproteins, of relevance because of LRP’s role in postprandial lipoprotein metabolism and association of the polymorphism with coronary artery disease. We found a postprandial decrease in LDLR mRNA abundance relative to the reference β-actin (BA) mRNA. The decreased LDLR/BA mRNA value was apparent at 1 h (P < 0.005) and decreased to 25% of baseline at 6 h (P < 0.005). The LRP/BA mRNA value was also lower at 6 h (16% decrease, P < 0.05). HMGCR mRNA expression was unchanged. C homozygotes for the C200T polymorphism had higher LDLR/BA values than T homozygotes (P = 0.01) and although plasma LDL cholesterol (LDL-C) concentrations decreased in the postprandial period (P < 0.002), the decrease was less in C than in T homozygotes (P < 0.05). This study constitutes the first observation, to our knowledge, of postprandial changes in LDLR and LRP mRNA expression. It documents immediate effects of a fatty meal on these mRNA as well as an LRP genotype effect on LDLR mRNA and LDL-C.

**Introduction**

The LDL receptor (LDLR) gene is downregulated by higher intracellular cholesterol (1,2), an effect first demonstrated in vitro. Dietary fat influences plasma lipids and the risk of coronary artery disease (CAD) and yet, despite the central role of the LDLR in lipid metabolism, there are few studies on the regulation of its cellular cholesterol (1,2), an effect first demonstrated in vitro. More immediate effects have not been studied and, to that end, we examined the acute effects of a single fat meal on the mRNA of these genes in circulating mononuclear cells. Several studies on these cells have examined the influence of diet on the mRNA expression of LDLR, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and LDLR-related protein (LRP) over several days or weeks (3,4). More immediate effects have not been studied and, to that end, we examined the acute effects of a single fat meal on the mRNA of these genes in circulating mononuclear cells. In addition, we examined the influence of a polymorphism in the LRP gene, namely the exon 22 C200T, both on postprandial metabolism and mRNA expression.

The role of the above 3 genes in lipid metabolism needs no further emphasis at this point, but study of the LRP exon 22 C200T polymorphism requires further explanation. It is relevant because we recently showed the presence of the C allele and C homozygosity to have a strong association with premature CAD (5) and Schulz et al. (6) reported the C200T polymorphism is part of an LRP haplotype, consisting of several loci in disequilibrium, which was also associated with myocardial infarction. Many polymorphisms have been identified in the LRP gene (7), but of 7 exonic polymorphisms we previously studied (5), this particular polymorphism showed the clearest relation with CAD. The association in both males and females (5), and the study by Schulz et al. (6), support the validity of the relation although the
basis for it remains unclear, especially because the C200T polymorphism, although exonic, does not result in an amino acid change. LRP is a member of the LDLR gene family, with diverse ligands involved in vascular physiology (8) and playing a particular role in chylomicron (CM) remnant (CR) clearance (9–11). We did not find an unequivocal influence of the polymorphism on fasting plasma lipid concentrations (5). However, in view of the role of LRP in the clearance of CR, lipoproteins that are considered by many to have an important role in the pathogenesis of CAD (12–14), it seemed plausible that the polymorphism may be associated with a postprandial increase in CR following a fatty meal. For example, the CC genotypes might delay the clearance of CR compared with that associated with the TT genotype, leading to higher postprandial concentrations in the C homozygotes. Hence, subjects homozygous for the C and T alleles of the LRP exon 22 polymorphism were selected for the study. This study can, therefore, be considered to encompass several novel aims: to determine the effect, over a 10-h period, of a single high-fat meal on the expression of the genes for LDLR, LRP, and HMGCR in circulating mononuclear cells; to assess any effect of the LRP exon 22 C200T polymorphism on plasma lipids immediately following the meal; and, implicitly, to examine the influence of the polymorphism on the expression of the target genes.

**Materials and Methods**

**Subjects**

We studied 20 male subjects, selected from those previously described (5), without evidence of CAD, on the basis of genotype, age-matching, and availability. Twelve were homozygous for the C and 8 for the T allele of the LRP exon 22 polymorphism. Ten of the CC subjects had the apo-lipoprotein (apo) E3/E3 genotype and 2 were E3/E2; 7 of the TT subjects were apo E3/E3 and 1 was apo E3/E2. The Ethics Committee of Royal Perth Hospital approved the study protocol and all subjects gave their informed consent.

Subjects abstained from alcohol for 48 h and fasted for 12 h. After obtaining fasting blood samples, they ingested, within 5 min, a high-fat load in the form of a milkshake and 2 capsules containing 105 μmol (equivalent to 100,000 IU) vitamin A as retinyl palmitate (RP; Retinol, Janssen-Cilag). The milkshake was prepared as described by Parhofer et al. (15), using retinyl acetate as an internal standard. Plasma, CM, and CR + VLDL fractions from any 1 subject were extracted and analyzed in the 1 analytical run.

**Lipid assays**

Plasma samples were assayed for TC, HDL-C, LDL-C, and TG, while CM and CR + VLDL fractions were assayed for TC, TG, and RP. TC, HDL-C, and TG concentrations were determined by enzymatic methods using Roche Diagnostics reagents on a Hitachi 917 analyzer. LDL-C concentrations were measured using a Direct LDL-C assay (Boehringer Mannheim) on the same instrument.

**RP extraction and analysis**

RP concentrations were determined by HPLC as described by Parhofer et al. (15), using retinyl acetate as an internal standard. Plasma, CM, and CR + VLDL fractions from any 1 subject were extracted and analyzed in the 1 analytical run.

**mRNA quantification**

**RNA isolation.** Duplicate samples of total RNA were preserved in Ultra spec reagent (Fisher Biotech). Briefly, they were extracted by a guanidinium thiocyanate–phenol–chloroform method. RNA pellets were washed twice with 75% ethanol (analytical grade, in diethylpyrocarbonate-treated water), suspended in RNase-free water (Qiagen), and stored at −70 to −80°C.

RNA integrity was also assessed by electrophoresis on an agarose gel. The yield of RNA from 278 samples was 21.0 ± 0.4 μg (mean ± SD).

**cDNA synthesis and quantification.** An aliquot (1 μg) of RNA was used for each reverse-transcription reaction. The RNA was denatured at 65°C for 5 min, cooled on ice, and added to a reaction mixture containing 2 μL of 500 mg/mL oligo(dT)15 primer (Promega), 2 μL of a 5 mM solution of deoxynucleoside triphosphate (dNTP) solution (1.25 mM/μL of each dNTP, Invitrogen), 2 μL of 10× RT buffer (Qiagen), 0.3 μL of 40 MUL RNAсин (Invitrogen), and 1 μL of 4.5 MUL Omniscript RT (Qiagen) in a total volume of 20 μL. The cDNA synthesis was performed in a Perkin Elmer Cetus DNA Thermal Cycler and was comprised of a 60-min incubation at 37°C and terminated by chilling to 4°C for 5 min.

A 5-μL aliquot of a 10-fold dilution of the cDNA was used for real-time quantitative PCR for all genes examined. This was added to 0.1-μL PCR strip tubes (Fisher Biotech) containing a 15 μL mix comprising: 2 μL of 10× PCR buffer (100 mMOL Tris-HCl, pH 8.3, 500 mMOL KCl, 0.1 g/L gelatin), 1.2 μL of 25 mMOL/L MgCl₂, 1 μL of 25 g/L bovine serum albumin, 1 μL of dNTP mix (final concentration 200 μmol/L for each dNTP), 1 μL of each of PCR primer (final concentration 0.5 μmol/L), 0.2 μL of 5 MUL Platinum Taq DNA polymerase, and 2 μL of a 5× concentration of SYBR Green I (Sigma). The PCR were carried out in real-time mode on a Rotor-Gene 3000 (Corbett Research) thermocycler.

The temperature program for PCR was as follows: 1 cycle of 95°C for 1 min, 35–40 cycles of 95°C for 10 s; 64°C for 15 s; and 72°C for 25 s. Fluorescence was measured after the extension step of each cycle at 72°C. The sequence of the primers used for amplification of the cDNA is documented in Supplemental Table 1.

**Statistical analyses**

Trends over time in the lipid and mRNA responses, and differences between the 2 genotypes, were tested using multiple linear mixed regression (on the log scale) with quadratic or cubic mixed models using SPSS (Insightful). The models incorporated adjustment for both BMI and apo E genotype. Where these comparisons indicated a significant response with time, or between genotypes, comparisons at specific time points were carried out using paired (for comparisons with baseline) or unpaired (between genotypes) t tests. Except where otherwise indicated, values in the text are means ± SEM and differences with P < 0.05 were
Concentration of plasma lipids, decreased from baseline for the 2–8-h and 4–8-h time intervals, elevated for a further 4 h. In contrast, plasma LDL-C and HDL-C Plasma TG concentrations increased significantly at 1 h, peaked Plasma lipid concentrations during the fat loading test.

ject were E3/E2 heterozygotes, the remaining being E3 homozygotes. an outlier and excluded all of this subject's data from analyses.

Lipids

**TABLE 2** Plasma lipid concentrations in men with CC and TT LRP genotypes at baseline and during the fat loading test

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Genotype group (n)</th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>10 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC + TT (19)</td>
<td>1.51 (0.54,4.20)</td>
<td>1.96* (0.73,5.30)</td>
<td>2.89* (1.02,8.18)</td>
<td>3.43* (0.95,12.47)</td>
<td>3.33* (0.92,12.03)</td>
<td>2.23* (0.56,8.02)</td>
<td>1.65 (0.47,5.80)</td>
</tr>
<tr>
<td></td>
<td>CC (12)</td>
<td>1.52 (0.89,2.53)</td>
<td>2.07 (0.99,3.74)</td>
<td>3.04 (1.38,5.83)</td>
<td>3.72 (1.53,7.26)</td>
<td>3.38 (1.95,0.01)</td>
<td>2.14 (0.94,5.44)</td>
<td>1.57 (0.73,3.83)</td>
</tr>
<tr>
<td></td>
<td>TT (7)</td>
<td>1.49 (0.29,7.64)</td>
<td>1.79 (0.40,8.07)</td>
<td>2.65 (0.56,12.55)</td>
<td>2.99 (0.40,22.31)</td>
<td>3.25 (0.38,28.12)</td>
<td>2.38 (0.32,17.85)</td>
<td>1.78 (0.30,10.41)</td>
</tr>
<tr>
<td>TG</td>
<td>CC + TT (19)</td>
<td>5.19 ± 0.18</td>
<td>5.37 ± 0.169</td>
<td>5.38 ± 0.17*</td>
<td>5.24 ± 0.17</td>
<td>5.24 ± 0.17</td>
<td>5.19 ± 0.17</td>
<td>5.22 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>CC (12)</td>
<td>5.30 ± 0.21</td>
<td>5.47 ± 0.18</td>
<td>5.47 ± 0.20</td>
<td>5.33 ± 0.19</td>
<td>5.32 ± 0.18</td>
<td>5.28 ± 0.19</td>
<td>5.31 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>TT (7)</td>
<td>5.01 ± 0.33</td>
<td>5.21 ± 0.30</td>
<td>5.22 ± 0.33</td>
<td>5.09 ± 0.35</td>
<td>5.09 ± 0.37</td>
<td>5.03 ± 0.32</td>
<td>5.08 ± 0.31</td>
</tr>
<tr>
<td>LDL-C</td>
<td>CC + TT (19)</td>
<td>3.39 ± 0.16</td>
<td>3.42 ± 0.14</td>
<td>3.25 ± 0.15*</td>
<td>3.03 ± 0.15*</td>
<td>3.01 ± 0.17*</td>
<td>3.16 ± 0.17*</td>
<td>3.32 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>CC (12)</td>
<td>3.55 ± 0.22</td>
<td>3.57 ± 0.19</td>
<td>3.41 ± 0.20</td>
<td>3.19 ± 0.20</td>
<td>3.24 ± 0.22</td>
<td>3.40 ± 0.22</td>
<td>3.48 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>TT (7)</td>
<td>3.13 ± 0.17</td>
<td>3.16 ± 0.17</td>
<td>2.97 ± 0.17</td>
<td>2.76 ± 0.18</td>
<td>2.61 ± 0.19</td>
<td>2.74 ± 0.20</td>
<td>3.05 ± 0.19</td>
</tr>
<tr>
<td>HDL-C</td>
<td>CC + TT (19)</td>
<td>1.19 ± 0.06</td>
<td>1.22 ± 0.07</td>
<td>1.19 ± 0.06</td>
<td>1.12 ± 0.06*</td>
<td>1.10 ± 0.06*</td>
<td>1.12 ± 0.07*</td>
<td>1.18 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>CC (12)</td>
<td>1.19 ± 0.07</td>
<td>1.22 ± 0.07</td>
<td>1.19 ± 0.07</td>
<td>1.11 ± 0.06</td>
<td>1.09 ± 0.05</td>
<td>1.12 ± 0.06</td>
<td>1.19 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>TT (7)</td>
<td>1.20 ± 0.14</td>
<td>1.22 ± 0.14</td>
<td>1.19 ± 0.14</td>
<td>1.14 ± 0.15</td>
<td>1.11 ± 0.15</td>
<td>1.11 ± 0.16</td>
<td>1.16 ± 0.15</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM or geometric means (95% CI). Overall time trend (for CC + TT): *, P < 0.002. Different from baseline: *, P ≤ 0.001; †, P ≤ 0.01. Overall time-dependent LRP genotype effect: †, P < 0.05. Differences from baseline for CC and TT subgroups are not shown.

RP, CM, and CR + VLDL. The TG concentration of the CM fraction increased significantly at 1 h and reached ~3–4 times the baseline value in the middle of the observation period (Supplemental Table 2). The changes in TG and cholesterol in the CR + VLDL fraction were small but significant (Supplemental Table 2). RP concentrations in plasma, CM, and CR + VLDL fractions increased significantly at 1 h and remained elevated above the baseline level for the remainder of the observation period (Supplemental Table 3). The RP concentration in plasma, and in the CM, and CR + VLDL fractions were not significantly different between CC and TT subjects (0.25 < P < 0.4). The TG and cholesterol concentrations of the CM and CR + VLDL fractions also did not differ between the genotype groups.

**RESULTS**

**Subject characteristics.** Baseline characteristics of the subjects divided according to their CC and TT genotype for the LRP exon 22 polymorphism did not differ (Table 1). One TT subject had a very high fasting plasma TG (13.7 mmol/L); we considered this an outlier and excluded all of this subject’s data from analyses. As mentioned in “Materials and Methods,” 2 CC and 1 TT subject were E3/E2 heterozygotes, the remaining being E3 homozygotes.

Plasma lipid concentrations during the fat loading test. Plasma TG concentrations increased significantly at 1 h, peaked at 4 h and similar concentrations were maintained for the 2–8-h and 4–8-h time intervals, respectively (Table 2). Although the 2 genotype subgroups did not differ in plasma HDL-C, TG, or TC concentrations, CC homozygotes had a significantly smaller decrease in plasma LDL-C postprandially than TT homozygotes (Table 2).

**TABLE 1** Baseline characteristics of men undergoing the oral fat loading test

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>LDL-C, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>TG, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>12</td>
<td>48 ± 3</td>
<td>27 ± 1</td>
<td>5.4 ± 0.5</td>
<td>1.2 ± 0.1</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>TT</td>
<td>7</td>
<td>28 ± 1</td>
<td>27 ± 1</td>
<td>3.6 ± 0.2</td>
<td>1.5 (0.9, 2.5)</td>
<td>1.5 (0.3, 7.6)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM or geometric means (95% CI). Overall time trend (for CC + TT): *, P < 0.002. Different from baseline: *, P ≤ 0.001; †, P ≤ 0.01. Overall time-dependent LRP genotype effect: †, P < 0.05. Differences from baseline for CC and TT subgroups are not shown.

**CONCLUSIONS**

Table 2 displays the data on LDLR, LRP, and HMGCR mRNA in 2 ways: the total or raw mRNA abundance and their abundance relative to a housekeeping or reference gene β-actin (BA). The former is the more common method of expression in the literature, but, in general, the results of the 2 approaches were consistent. Results for the mRNA studies were not available for 2 subjects, a C and a T homozygote, due to excessive RNA degradation during the isolation procedure.

In response to the fat meal, LDLR and LDLR mRNA expression decreased without change in HMGCR mRNA expression (Table 3). There was a significant postprandial decrease in LDLR relative to BA mRNA (LDLР/BA) apparent as early as 1 h. Collectively, the lowest level attained, a 25% decrease compared with the baseline value, occurred 6 h postprandially. The raw mRNA LDLR data showed similar behavior, also reaching a nadir, corresponding to a 21% decrease, 6 h postprandially. The fat meal also resulted in significant decreases in LDLР/BA and raw LDLR mRNA expression, which were similar.

The maximum decreases in LDLР/BA and raw LDLR mRNA were similar in magnitude to those for LDLR mRNA (21–25%) but occurred later at 8 and 10 h.

Raw LDLR mRNA abundance tended to be greater in CC subjects than in TT subjects (P = 0.15), whereas the LDLR/BA was significantly higher in CC subjects at baseline and for the entire study period (Table 3). Whereas CC subjects had a significantly higher LDLR mRNA expression, the postprandial changes in LDLR mRNA did not differ between the 2 genotype subgroups. For example, the nadir in LDLР/BA occurred at the 6-h
The postprandial state and LDL-receptor mRNA

time point in both groups and was of similar magnitude, representing decreases of 26% and 25% in CC and TT subjects, respectively. The CC and TT subjects did not differ in LRP mRNA or LRP/BA expression (Table 3).

Finally, and in contrast to results on LDLR and LRP mRNA, there was no evidence of any changes in HMGCR mRNA expression over the postprandial period nor of any LRP genotype effect (not shown).

Discussion

Our study encompassed several novel aims. It is the first observation, to our knowledge, of acute postprandial changes in LDLR, LRP, or HMGCR mRNA expression in circulating mononuclear cells in response to a high-fat meal. It also examined the effect of the LRP exon 22 C200T polymorphism on plasma lipids immediately following the meal, which, implicitly, enabled examination of the influence of the polymorphism on the expression of the aforementioned mRNAs in mononuclear cells.

Although the results on the mRNA of the target genes proved to be of greater interest, the influence of the LRP polymorphism on postprandial lipoprotein metabolism will be discussed first.

Influence of the LRP polymorphism on postprandial lipids.

Because of the association of the LRP C200T polymorphism with premature CAD and the role of LRP in the clearance of CR, we considered it plausible that the polymorphism may be associated with delayed clearance of CR in the postprandial period. In examining C and T homoyzogotes, we also took account of variables that might influence postprandial lipoprotein clearance. All subjects were male Caucasians, within a small age range, abstained from alcohol for 48 h, and consumed a normal western diet. Apo E polymorphisms, including the E2 and E4 alleles, reportedly affect postprandial lipoprotein metabolism (16–21). Their possible influence was considered by using multivariate mixed models for adjustment.

Dietary fat is assembled into TG-rich CM and secreted into the circulation before hydrolysis by lipoprotein lipase to CR (22). Because it is difficult to separate CM and CR from VLDL and VLDL remnants, studies examining postprandial changes in CM and CR often use retinol or RP as a marker for these intestinally derived lipoproteins. Following absorption, RP is secreted from the intestine as a component of CM and largely remains with the particles as they are metabolized to CR. RP is therefore considered a better marker for CM and CR than plasma TG, which also reflects liver-produced VLDL and VLDL remnants (23). However, the concentrations of RP, in plasma as well as in CM and CR + VLDL fractions, were not significantly higher in CC than TT subjects. Nevertheless, the data do not completely exclude a difference in CM and CR clearance between the 2 LRP genotype subgroups, because the high degree of between-individual variation limited statistical power. On the other hand, we observed a smaller postprandial decrease in plasma LDL-C in C homozygotes.

mRNA studies: influence of the high-fat meal. The mRNA of the target genes was measured in human mononuclear cells, which have also been examined in more prolonged dietary studies (3,4,24). It is relevant that the expression of LDLR and HMGCR mRNA in the general population of circulating mononuclear cells from normal subjects correlated with that of the corresponding mRNA in the liver (25). As for liver cells, the synthesis of sterols in mononuclear cells was found to depend on HMGCR activity (26), and the binding of LDL, reflecting LDLR activity, was upregulated by incubation in lipoprotein-deficient medium (27). It therefore appears that, in humans, the coordinate regulation of HMGCR and of LDLR is similar in liver and mononuclear cells, the latter being more accessible for studies on human subjects.

Our results indicate that LDLR mRNA expression decreased in both LRP genotype subgroups during the postprandial period. To our knowledge, no other studies have examined the immediate effect on gene expression of an acute dietary intervention in man, although the influence of a change in diet has been studied. Vidon et al. (4) found a 3-wk high-fat diet had little influence on the LDLR mRNA of peripheral mononuclear cells. In contrast, Boucher et al. (3) found that the LDLR mRNA expression of circulating mononuclear cells in men increased in response to 4 d of consuming a low-cholesterol, low-fat diet and decreased when the fat-restricted diet was supplemented with cholesterol. The present study has considerably extended that observation. Our finding is analogous to the early observation on human

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**TABLE 3** LDLR and LRP mRNA expression in men with CC and TT LRP genotypes during the fat loading test

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Genotype group (n)</th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>10 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR</td>
<td>CC + TT (17)</td>
<td>1930 ± 140</td>
<td>1780 ± 170</td>
<td>1720 ± 190</td>
<td>1620 ± 150</td>
<td>1520 ± 130</td>
<td>1610 ± 120</td>
<td>1720 ± 120</td>
</tr>
<tr>
<td></td>
<td>TT (6)</td>
<td>1670 ± 190</td>
<td>1380 ± 110</td>
<td>1290 ± 120</td>
<td>1470 ± 140</td>
<td>1240 ± 160</td>
<td>1390 ± 180</td>
<td>1550 ± 190</td>
</tr>
<tr>
<td>LRP</td>
<td>CC + TT (17)</td>
<td>1300 ± 100</td>
<td>1300 ± 150</td>
<td>1300 ± 180</td>
<td>1200 ± 130</td>
<td>1100 ± 120</td>
<td>1000 ± 79</td>
<td>1000 ± 87</td>
</tr>
<tr>
<td></td>
<td>CC (11)</td>
<td>1300 ± 150</td>
<td>1400 ± 210</td>
<td>1400 ± 260</td>
<td>1300 ± 200</td>
<td>1200 ± 170</td>
<td>1100 ± 120</td>
<td>1000 ± 130</td>
</tr>
<tr>
<td></td>
<td>TT (6)</td>
<td>1200 ± 110</td>
<td>1100 ± 140</td>
<td>1000 ± 100</td>
<td>1200 ± 140</td>
<td>980 ± 110</td>
<td>930 ± 77</td>
<td>950 ± 100</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM.
2 LDLR/BA and LRP/BA results have been multiplied by 10⁵. Overall time trend (for CC + TT): $, P = 0.001; *, P < 0.02. Different from baseline: *, P < 0.005; $, P ≤ 0.025; ¦, P < 0.05. Overall time-independent LRP genotype effect: **, P = 0.01. Different between CC and TT genotypes: 1, P < 0.01; 1, P < 0.025; 1, P < 0.05. Differences from baseline for CC and TT subgroups are not shown.
mononuclear cells, in vivo, of a similarly timed, decreased expression of LDLR mRNA upon exposure to LDL (2). However, it is noteworthy that, in our study, the postprandial increases in plasma TC and CM cholesterol were small, whereas LDL-C decreased slightly. The decreased LDLR mRNA was, presumably, due to an intracellular effect subsequent to the influx of dietary fat or cholesterol. Further studies to elucidate the precise mechanism underlying these mRNA changes are required, but investigations in other species have found clear evidence that LDL-receptor expression can be influenced not only by cholesterol but also by fatty acids (28,29); PUFA and SFA having opposite effects.

The trend for lower LRP mRNA expression became significant during the later part of the postprandial period. There are no other comparable studies, to our knowledge, but Vidon et al. (4) reported LRP mRNA in circulating mononuclear cells to be higher in subjects who consumed a high-fat diet for 3 wk compared with an isocaloric high-carbohydrate diet, while Boucher et al. (3) reported a decrease in response to 4 d of consuming a low-cholesterol, low-fat diet and an increase after reintroduction of cholesterol to the diet. The acute response we found is contrary to these observations, (3,4) but, whereas little is known of the regulation of the LRP gene, the concordant behavior of LDLR and LRP mRNA in our study indicates that the immediate regulation of the 2 genes, in response to a fat load, are directionally similar, and supports previous suggestions that the roles of these 2 receptors in lipoprotein uptake by cells may be complementary (9–11).

The mRNA for HMGCR was unaffected by consumption of the fat meal. In contrast, Vidon et al. (4) found HMGCR mRNA expression in mononuclear cells was lower in subjects consuming a high-fat diet for 3 wk compared with those consuming a high-carbohydrate diet; Boucher et al. (3) found the expression was higher in subjects consuming a diet low in cholesterol and fat and decreased when the fat-restricted diet was supplemented with cholesterol. Those findings are consistent with the coordinate regulation of HMGCR and LDLR genes by cholesterol (1) and with the mRNA expression of HMGCR and LDLR in mononuclear leukocytes generally being directly correlated (25). One other study in men found that HMGCR enzyme activity in mononuclear cells decreased within 2 h of a single high-cholesterol meal (24). Unless this different result relates to the nature of the meal, reconciliation of this finding on enzyme activity with our mRNA result would imply that other aspects of HMGCR regulation are required, but investigations of the few dietary studies available but consistent with coordinate regulation of the LDLR and LRP genes. There was no clear effect of the LRP exon C200T polymorphism on most plasma lipids postprandially, but, compared with their T homozygote counterparts, C homozygotes had a smaller decrease in plasma LDL-C concentrations. The higher LDLR mRNA expression in C homozygotes may represent partial compensation for defective LRP function.

**mRNA studies: influence of LRP genotype.** Broadly, the mRNA data indicated that subjects with the CC genotype had a higher expression of LDLR mRNA in mononuclear cells than did TT subjects. On the other hand, the postprandial changes in the mRNA did not differ between these 2 genotype subgroups. It is presently unclear why the LRP gene polymorphism should influence LDLR gene expression, but there is evidence that the roles of LDLR and LRP are coordinated in the cellular binding and uptake of remnant particles (9–11). One possibility is that inheritance of the C allele is associated with diminished LRP function and results in a compensatory increase in the expression of LDLR. There is no other information available concerning the function of LRP resulting from the C200T polymorphism. However, it should be noted that the polymorphism does not result in an amino acid substitution; the influence of the polymorphism is therefore most likely due to linkage to other more functional polymorphisms in the LRP gene. Finally, although we did not substantiate defective remnant clearance in CC subjects, it may be that any defect was partly compensated by the increase in LDLR gene expression.

This study provided novel evidence of an early decrease in LDLR mRNA in men and a later decrease in LRP mRNA in circulating mononuclear cells during the postprandial period. The relatively rapid change in LDLR mRNA expression is consistent with several longer term dietary intervention studies and more basic observations on the regulation of LDLR gene expression by lipids. Less is known about the regulation of the LRP gene. The postprandial decrease in LRP mRNA is contrary to the results of the few dietary studies available but consistent with coordinate regulation of the LDLR and LRP genes. There was no clear effect of the LRP exon C200T polymorphism on most plasma lipids postprandially, but, compared with their T homozygote counterparts, C homozygotes had a smaller decrease in plasma LDL-C concentrations. The higher LDLR mRNA expression in C homozygotes may represent partial compensation for defective LRP function.

**Literature Cited**


The postprandial state and LDL-receptor mRNA