Normal Oxidative Stress and Enhanced Lipoprotein Resistance to In Vitro Oxidation in Hypertriglyceridemia
Effects of Bezafibrate Therapy


Abstract—Although there is evidence that hyperlipidemia and predominance of small dense low density lipoproteins (LDLs) are associated with increased oxidative stress, the oxidation status in patients with hypertriglyceridemia (HTG) has not been studied in detail. Therefore, we studied urinary levels of 2,3-dinor-5,6-dihydro-8-isoprostaglandin F$_{2a}$ and susceptibility of very low density lipoproteins (VLDLs) and LDLs to oxidation ex vivo in 18 patients with endogenous HTG and 20 matched control subjects. In addition, the effects of 6 weeks of bezafibrate therapy were assessed in a double-blind, placebo-controlled, crossover trial. Urinary levels of 2,3-dinor-isoprostanes were similar in the HTG and normolipidemic group. Bezafibrate caused an increase in 8-isoprostaglandin F$_{2a}$ (762±313 versus 552±245 ng/24 h for bezafibrate and placebo therapy, respectively; $P=0.03$), whereas 2,3-dinor-5,6-dihydroxy-8-isoprostaglandin F$_{2a}$ levels tended to be increased (1714±761 versus 1475±606 ng/24 h for bezafibrate and placebo therapy, respectively; $P=0.11$). VLDLs and LDLs were more resistant to copper-induced oxidation in patients with HTG than in control subjects. Bezafibrate reversed the oxidation resistance to the normal range. In conclusion, these results indicate the following: (1) HTG is associated with normal in vivo oxidative stress and enhanced ex vivo resistance of lipoproteins to oxidation. (2) Bezafibrate reduces the resistance of lipoproteins to copper-induced oxidation and enhances oxidative stress in HTG patients. (Arterioscler Thromb Vasc Biol. 2000;20:2434-2440.)

Key Words: hypertriglyceridemia ■ LDL oxidation ■ VLDL oxidation ■ F$_{2}$-isoprostanes ■ bezafibrate

Hypertriglyceridemia (HTG), an independent risk factor for cardiovascular disease, is characterized by elevated levels of VLDLs, low levels of HDL cholesterol (HDL-C), small dense LDLs, and insulin resistance. Small dense LDL particles have been suggested to be prone to oxidative modification and may contribute to the increased cardiovascular risk in HTG. Several forms of hyperlipidemia have been shown to be associated with increased oxidative stress; however, the oxidation status in HTG patients has not been studied in detail.

Ex vivo, the peroxidation process can be mimicked by incubating isolated lipoproteins with the pro-oxidant Cu$^{2+}$ and by measuring the production of conjugated dienes from polyunsaturated fatty acids (PUFAs). Earlier, we detected changes in susceptibility of lipoproteins to oxidation after supplementation with fish oil. Direct measurement of oxidation products is considered to be more indicative of in vivo oxidative stress. F$_{2}$-Isoprostanes, chemically stable end products of lipid peroxidation, have emerged as a promising marker of oxidative stress. In vitro and in vivo studies have demonstrated that oxidative stress results in a dose-dependent elevation of F$_{2}$-isoprostane levels. Previous studies have demonstrated increased F$_{2}$-isoprostane levels in smokers, diabetics, and hypercholesterolemic patients. The present study was undertaken to compare urinary levels of F$_{2}$-isoprostanes and susceptibility of VLDLs and LDLs to oxidation in vitro between patients with endogenous HTG and control subjects. In addition, the effects of triglyceride-lowering therapy by bezafibrate were studied.

Methods

Patients and Control Subjects

The study population consisted of 18 unrelated patients with endogenous HTG who were recruited from our lipid clinic. All patients received personal dietary advice. The diagnosis of endogenous HTG was based on the means of 2 fasting blood samples obtained after the dietary period of at least 8 weeks. The diagnostic criteria for endogenous HTG were as follows: total serum triglyceride level >4.0 mmol/L, VLDL cholesterol (VLDL-C) level >1.0 mmol/L, total cholesterol (TC) level >5.0 mmol/L, and LDL cholesterol (LDL-C) level >3.0 mmol/L. The diagnosis of endogenous HTG was based on a fasting blood sample obtained after a dietary period of at least 8 weeks. The diagnostic criteria for endogenous HTG were as follows: total serum triglyceride level >4.0 mmol/L, VLDL cholesterol (VLDL-C) level >1.0 mmol/L, total cholesterol (TC) level >5.0 mmol/L, and LDL cholesterol (LDL-C) level >3.0 mmol/L. The patients and control subjects were studied in a double-blind, placebo-controlled, crossover trial. Urinary levels of F$_{2}$-isoprostanes (8-isoprostaglandin F$_{2a}$) and susceptibility of very low density lipoproteins (VLDLs) and LDLs to oxidation ex vivo in 18 patients with endogenous HTG and 20 matched control subjects. In addition, the effects of 6 weeks of bezafibrate therapy were assessed in a double-blind, placebo-controlled, crossover trial. Urinary levels of F$_{2}$-isoprostanes were similar in the HTG and normolipidemic group. Bezafibrate caused an increase in 8-isoprostaglandin F$_{2a}$ (762±313 versus 552±245 ng/24 h for bezafibrate and placebo therapy, respectively; $P=0.03$), whereas 2,3-dinor-5,6-dihydroxy-8-isoprostaglandin F$_{2a}$ levels tended to be increased (1714±761 versus 1475±606 ng/24 h for bezafibrate and placebo therapy, respectively; $P=0.11$). VLDLs and LDLs were more resistant to copper-induced oxidation in patients with HTG than in control subjects. Bezafibrate reversed the oxidation resistance to the normal range. In conclusion, these results indicate the following: (1) HTG is associated with normal in vivo oxidative stress and enhanced ex vivo resistance of lipoproteins to oxidation. (2) Bezafibrate reduces the resistance of lipoproteins to copper-induced oxidation and enhances oxidative stress in HTG patients. (Arterioscler Thromb Vasc Biol. 2000;20:2434-2440.)
and LDL cholesterol (LDL-C) level <4.5 mmol/L. Additional exclusion criteria were a history of cardiovascular disease, homozygosity for apoE2, secondary hyperlipidemia (renal, liver, or thyroid disease; fasting glucose >7.0 mmol/L; and alcohol consumption >40 g/d), and the use of lipid-lowering drugs. Twenty normolipidemic age- and sex-matched control subjects were recruited in response to a newspaper advertisement. None of the participants took vitamin supplementation or aspirin during the study, whereas 5 HTG patients and 1 control subject took antihypertensive drugs, which were continued during the study.

Study Design

The patients were randomized to receive, in a double-blind crossover fashion, bezafibrate (400 mg once daily) or placebo for 6 weeks. The 2 treatment periods were separated by a 6-week washout period. Before and at the end of each treatment period, fasting venous blood samples were obtained. From the control subjects, fasting blood samples were obtained at baseline. Urinary F₃-isoprostane levels were determined in 24-hour urine samples from the HTG patients, obtained at the end of both treatment periods, and in overnight urine samples from the control group. Informed consent was obtained from each participant, and the protocol was approved by the Medical Ethics Committee of our institution.

Lipid and Lipoprotein Analyses

Serum was obtained after centrifugation at 1500g for 15 minutes at room temperature. Three milliliters of fresh serum was ultracentrifuged for 15 hours at 232,000 g at 15°C in a TL-100 tabletop ultracentrifuge, with use of a TLA-100.3 fixed-angle rotor (Beckman). Ultracentrifugation yielded density fractions of <1.006 and 1.006 to 1.25 g/mL, designated as the VLDL and HDL fraction, respectively. HDL-C was measured in the LDL-HDL fraction after precipitation with phosphotungstic acid and MgCl₂. Serum was obtained after centrifugation at 1500 g at 15°C in a TL-100 tabletop ultracentrifuge, with use of a TLA-100.3 fixed-angle rotor (Beckman). Serum triglyceride, total cholesterol, phospholipid, and free cholesterol concentrations were measured enzymatically in the isolated HDL, LDL, and VLDL fractions by use of commercially available kits (Boehringer-Mannheim). Cholesterol ester content was calculated by subtracting the amount of free cholesterol from the concentration of total cholesterol. Protein was determined by the method of Lowry et al. Lipid diameter was determined by photon correlation spectroscopy (Malvern Instruments). LDL particle size was analyzed by gradient gel electrophoresis.

Fatty acid composition was determined by gas chromatography after methylation of the fatty acids. The total number of double bonds in VLDL and LDL equaled the relative content of each fatty acid with ≥2 double bonds times its number of double bonds. Mono-unsaturated fatty acids (MUFA) were not included in the calculation because they are less susceptible to oxidation. Vitamin E (α-, γ- and δ-tocopherol) was assessed by high-performance liquid chromatography with UV detection at 292 nm.

Measurements of F₃-Isoprostanes

Urine samples were stored in 5 mL aliquots at −80°C. 8-Isoprostaglandin F₃α (iPF₃α-III) levels were determined by use of gas chromatography–tandem mass spectrometry. In addition, 2,3-dinor-5,6-dihydro-8-isoprostaglandin F₃α (2,3-dinor-5,6-dihydro-iPF₃α-III), the major urinary metabolite of iPF₃α-III, was measured by use of the same method. Deuterium-labeled iPF₃α-III (1 ng/mL, Cayman Chemical) and ¹⁸O-labeled est-2,3-dinor-5,6-dihydro-iPF₃α-III (1 ng/mL) were used as internal standards. Inter assay variance was 6.8% for iPF₃α-III and 6.0% for 2,3-dinor-5,6-dihydro-iPF₃α-III; intra-assay variance was 6.5% for iPF₃α-III and 4.0% for 2,3-dinor-5,6-dihydro-iPF₃α-III. To compare the quantities of F₃-isoprostanes excreted in urine between control subjects and HTG patients, F₃-isoprostanes were corrected for creatinine excretion in urine. Because bezafibrate is known to increase urinary creatinine excretion, absolute levels of both F₃-isoprostanes in 24-hour urine were compared between HTG patients on placebo and on bezafibrate therapy. In 3 of 18 HTG patients, 24-hour urine collection was incomplete; therefore, 15 paired data were available for absolute F₃-isoprostane analysis.

Oxidation of VLDL and LDL

Fasting venous blood, drawn in EDTA tubes, was centrifuged within 1 hour for 15 minutes at 1500g at 4°C. The plasma samples were brought to a final concentration of 10% (wt/vol) sucrose, capped under nitrogen, submerged in liquid nitrogen, and stored at −80°C. The samples were analyzed within 6 months. Lipoproteins were separated by ultracentrifugation at 4°C by use of standard methods, with the use of 0.1 mg/mL LDL and 40 µg/mL CuSO₄, was assayed by serial measurement of the conjugated dienes formed. The same procedure was applied to VLDLs, but with a lower protein concentration (0.03 mg/mL) to avoid turbidity. The formation of conjugated dienes was measured by monitoring the change in absorbance at 234 nm in a spectrophotometer. Lag time and propagation rate were determined as previously described. The total quantity of conjugated dienes was expressed in nanomoles formed per milligram of VLDL or LDL protein. The VLDL and LDL samples of a control subject and a patient, during placebo and bezafibrate therapy, were oxidized on the same day in 3 oxidation runs.

Statistical Analyses

Results are presented as mean±SD. Differences between controls and patients were calculated by the Mann-Whitney U test. Differences in categorical variables between patients and controls were assessed by the Fisher exact test. Differences between the patient group on placebo and bezafibrate therapy were evaluated pairwise by the Wilcoxon paired signed rank test. Correlation analysis was performed by Spearman rank correlation analysis. A value of P<0.05 was considered significant.

Results

Patient Characteristics

As shown in Table 1, the patient and control groups were comparable with regard to age, sex, hypertension, and smoking habits. The HTG patients had a higher body mass index. Serum triglyceride levels were 13-fold higher in the HTG patients than in the control subjects. Both LDL-C and HDL-C levels were lower in the patient group, whereas VLDL-C concentrations were markedly elevated and accounted for the elevation in total serum cholesterol concentrations (Table 2).

Effect of Bezafibrate Therapy on Serum Lipids and Lipoproteins

All subjects concluded the study without any side effects. No significant changes in body weight occurred. Treatment with placebo had no effect on serum lipid levels (data not shown). Therefore, only the values obtained at the end of both treatment periods were compared. Bezafibrate therapy caused reductions in serum triglyceride, cholesterol and VLDL-C levels and increments in LDL-C and HDL-C levels (Table 2).
Levels of urinary F_2-isoprostanes were similar in HTG and HDL-C, mmol/L 1.32 ±0.29* 0.72 ±0.18* 4.37 ±0.84§ 6
LDL-C, mmol/L 3.52 ±0.87† 2.67 ±0.64 3.58 ±0.84§ 6
0.12±0.29* 0.72±0.13 0.91±0.13†
Values are mean±SD based on triplicate measurements.
TG indicates total triglycerides; TC, total cholesterol.
*P<0.001, †P<0.01, ‡P<0.001, and §P<0.01 vs HTG group on placebo therapy.

F_2-Isoprostanes Levels and Lipoprotein Oxidation Parameters

Levels of urinary F_2-isoprostanes were similar in HTG patients and control subjects (iPF_2r-III, 99±45 versus 103±52 nmol/mol creatinine; 2,3-dinor-5,6-dihydro-iPF2_r-III, 281±134 versus 260±111 nmol/mol creatinine for HTG patients and controls, respectively). Bezafibrate caused an increase in urinary iPF_2r-III levels (762±313 versus 552±245 ng/24 h for bezafibrate and placebo therapy, respectively; P=0.03), whereas urinary 2,3-dinor-5,6-dihydro-iPF_2r-III levels tended to be increased (1714±761 versus 1475±606 ng/24 h for bezafibrate and placebo, respectively; P=0.11).

Positive correlations were observed between urinary levels of iPF_2r-III and 2,3-dinor-5,6-dihydro-iPF_2r-III expressed per mole creatinine in the control group (r=0.781, P<0.001) and between urinary iPF_2r-III and 2,3-dinor-5,6-dihydro-iPF_2r-III concentrations in the patient group on placebo (r=0.70, P<0.001) and bezafibrate therapy (r=0.675, P<0.001). No significant correlations were observed between F_2-isoprostanes and any of the lipids and lipoproteins.

VLDL Oxidation

In the patient group, the lag time of VLDL oxidation was significantly higher and the propagation rate of VLDL oxidation was significantly lower than in the control group (Table 3, Figure 1). However, the maximum diene formation in the HTG group was higher than in the control group. Bezafibrate caused significant reductions in lag time and maximum diene production, whereas the propagation rate was unaffected.

LDL Oxidation

Oxidation characteristics of LDL paralleled that of VLDL. In the patient group, the lag time of LDL oxidation was significantly higher and the propagation rate of LDL oxidation was significantly lower than in the control group (Table 3, Figure 1). The maximum diene formation in the patient group was significantly lower than in the control group. On bezafibrate therapy, the lag time of LDL oxidation decreased, maximum diene formation increased, and the propagation rate did not change.

Determinants of Ex Vivo Oxidation Parameters

VLDL Oxidation

VLDL size and composition differed markedly between the patient and control groups (Table 4). VLDL particle size correlated with lag time (r=0.65, P<0.001) and maximum diene formation (r=0.52, P=0.001). The large VLDL particle size in HTG patients was associated with an increased vitamin E quantity, which decreased on bezafibrate therapy.

VLDL of HTG patients on placebo contained more saturated fatty acids (SFAs) and PUFAs than did VLDL of the control group (Table 4). However, the molar ratio of PUFA to SFA was lower in the patient group on placebo (0.71±0.20) than in the control group (1.00±0.31, P=0.03). The contributions of the individual fatty acids are presented in Figure 2. HTG VLDL contained more palmitic acid (C16:0) and stearic acid (C18:0) and less γ-linolenic acid (C18:3 ω6), docosapentaenoic acid (C22:5 ω3), and docosahexaenoic acid (C22:6 ω3) than did control VLDL. Bezafibrate therapy significantly altered neither the molar ratio of PUFA to SFA nor the number of double bonds in VLDL.

In accordance with a previous report, the lag time of VLDL oxidation was inversely correlated with the total number of double bonds (pooled data, r=−0.72, P<0.001). The bezafibrate-induced change in lag time correlated inversely with the change in double bonds (r=−0.828, P<0.001). The propagation rate of VLDL oxidation correlated positively with the total number of double bonds in VLDL (pooled data, r=0.78, P<0.001).

TABLE 3. In Vitro Lipoprotein Oxidizability and Vitamin E Levels

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>HTG Patients (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Bezafibrate</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time, min</td>
<td>143±30*</td>
<td>226±43</td>
</tr>
<tr>
<td>Propagation rate, nmol diene·min⁻¹·mg protein⁻¹</td>
<td>12.0±3.1†</td>
<td>10.0±3.0</td>
</tr>
<tr>
<td>Maximal dienes, nmol diene·mg protein⁻¹</td>
<td>1098±227‡</td>
<td>1459±397</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time, min</td>
<td>89±7*</td>
<td>108±17</td>
</tr>
<tr>
<td>Propagation rate, nmol diene·min⁻¹·mg protein⁻¹</td>
<td>12.1±1.1*</td>
<td>9.5±1.8</td>
</tr>
<tr>
<td>Maximal dienes, nmol diene·mg protein⁻¹</td>
<td>539±39*</td>
<td>406±50</td>
</tr>
<tr>
<td>Plasma vitamin E, μmol/L</td>
<td>26.9±5.7*</td>
<td>93.3±33.5</td>
</tr>
</tbody>
</table>

Values are mean±SD based on triplicate measurements.
*P<0.001, †P<0.05, ‡P<0.01, §P<0.05, ¶P<0.01, and ||P<0.001 vs HTG group on placebo therapy.
LDL Oxidation

The LDL particles of the HTG patients were significantly smaller (23.5 ± 0.6 nm) than those of the control subjects (25.2 ± 0.7 nm, P < 0.001; Table 4), and they increased in size on bezafibrate therapy (24.4 ± 1.1 nm, P = 0.003). Neither LDL size nor the vitamin E content of LDL was correlated with any of the oxidation parameters.

Like VLDL, LDL of HTG patients was enriched in SFA compared with that of control subjects (Table 4). Accordingly, the ratio of PUFA to SFA in LDL of the patient group increased.

Table 4. Lipoprotein Size and Composition

<table>
<thead>
<tr>
<th>VLDL</th>
<th>HTG Patients</th>
<th>LDL</th>
<th>HTG Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Placebo</td>
<td>Placebo</td>
</tr>
<tr>
<td>Particle size, nm</td>
<td>40.9 ± 3.0*</td>
<td>62.2 ± 17.6</td>
<td>44.4 ± 4.3§</td>
</tr>
<tr>
<td>FC, mass (%)</td>
<td>4.1 ± 1.4</td>
<td>5.4 ± 2.2</td>
<td>4.3 ± 1.1</td>
</tr>
<tr>
<td>CE, mass (%)</td>
<td>6.3 ± 2.1†</td>
<td>9.1 ± 2.5</td>
<td>8.1 ± 2.8</td>
</tr>
<tr>
<td>TG, mass (%)</td>
<td>60.4 ± 4.3</td>
<td>61.8 ± 6.5</td>
<td>61.8 ± 4.9</td>
</tr>
<tr>
<td>PL, mass (%)</td>
<td>15.2 ± 2.2</td>
<td>14.4 ± 2.0</td>
<td>15.1 ± 1.4</td>
</tr>
<tr>
<td>Prot, mass (%)</td>
<td>14.0 ± 2.6*</td>
<td>9.3 ± 2.3</td>
<td>10.7 ± 2.5</td>
</tr>
<tr>
<td>Vitamin E/protein, μmol/g</td>
<td>19.1 ± 12.9</td>
<td>30.2 ± 13.6</td>
<td>22.4 ± 12.3</td>
</tr>
<tr>
<td>Total FAs, μmol/mg protein</td>
<td>7.3 ± 2.3*</td>
<td>15.0 ± 5.0</td>
<td>12.6 ± 2.6</td>
</tr>
<tr>
<td>SFAs, μmol/mg protein (%)</td>
<td>2.5 ± 0.8* (34)</td>
<td>5.9 ± 1.9 (39)</td>
<td>5.0 ± 0.9 (40)</td>
</tr>
<tr>
<td>MUFA, μmol/mg protein (%)</td>
<td>2.4 ± 0.8* (33)</td>
<td>4.9 ± 1.8 (33)</td>
<td>4.4 ± 1.1 (35)</td>
</tr>
<tr>
<td>PUFAs, μmol/mg protein (%)</td>
<td>2.4 ± 0.9* (33)</td>
<td>4.1 ± 1.6 (28)</td>
<td>3.2 ± 0.9 (25)</td>
</tr>
<tr>
<td>Double bonds, %</td>
<td>85.0 ± 16.9‡</td>
<td>69.9 ± 12.1</td>
<td>67.6 ± 11.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD. FC, CE, TG, PL, and Prot represent relative weights of unesterified cholesterol, cholesteryl esters, triglycerides, phospholipids, and protein per particle, respectively; FAs, fatty acids.

*P < 0.001, †P < 0.01, ‡P < 0.05, §P < 0.001, ||P < 0.01, and ¶P < 0.05 vs corresponding HTG group on placebo therapy.

Figure 1. Ex vivo lipoprotein oxidizability. For VLDL (A) and LDL (B), lag times and propagation rates are shown. Groups A, B, and C represent HTG patients on placebo, HTG patients on bezafibrate, and control subjects, respectively.
(1.45±0.45) was lower than the ratio in the control group (1.67±0.23, P=0.02). HTG LDL showed a tendency to more myristic acid (14:0), palmitic acid (C16:0), and stearic acid (C18:0) than did control LDL (Figure 2). However, these differences did not reach statistical significance. The low contribution of PUFA in HTG LDL was mainly attributable to linoleic acid (C18:2 ω6). Bezafibrate therapy affected neither the ratio of PUFA to SFA in LDL nor the number of double bonds in LDL.

The total number of double bonds in LDL correlated inversely with lag time of LDL oxidation (pooled data, r = −0.65, P<0.001) and positively with the propagation rate of LDL oxidation (pooled data, r = 0.61, P=0.001).

Discussion

The present study was designed to gain more insight into the oxidation status of patients with HTG. Therefore, in vivo oxidation products and ex vivo oxidation behavior of lipoproteins were assessed. Our finding that urinary levels of 2 F2-isoprostanes were similar between HTG patients and matched normolipidemic control subjects provides compelling evidence that oxidative stress is not increased in HTG patients. This conclusion is strengthened by our observation that vigorous triglyceride-lowering therapy by bezafibrate did not lower F2-isoprostane levels. This finding is in sharp contrast with hypercholesterolemia, which has been associated with increased levels of F2-isoprostanes.11 Possibly the lower ratio of PUFA to SFA in HTG protects against the increased oxidative stress that is associated at least with elevated plasma cholesterol levels,11 which are also observed in HTG patients. On the other hand, the plasma cholesterol levels are only mildly elevated in HTG patients compared with the hypercholesterolemic patients described by Reilly et al11 and are distributed differently among lipoproteins, leaving open the possibility that there is no increased oxidative stress in HTG.

When the susceptibility to oxidation in vitro of separate lipoproteins in HTG was studied, it was found that VLDL and LDL demonstrated increased oxidation resistance (lag times) and lower oxidation rates (propagation rates) in the HTG group than in the control group. These results indicate an increased resistance to oxidative stress in vitro in HTG. The maximum diene production may reflect the quantity of oxidizable lipid per lipoprotein, resulting in a higher maximum for VLDL and a lower maximum for LDL in the HTG group compared with the control group.

The vitamin E contents of the isolated lipoproteins paralleled lipoprotein size. Although vitamin E is regarded as a strong lipoprotein-associated antioxidant, no significant correlations were noted between vitamin E content and in vitro oxidation parameters in either the VLDL or the LDL fraction. The latter is in accordance with reports showing the same results in unsupplemented healthy control groups.19,21 It has been demonstrated that the degree of unsaturation of fatty acids is a more important determinant of the susceptibility of lipoproteins to oxidation than is their vitamin E content.22,23
Previously, we observed strong correlations between the number of double bonds in the lipoprotein–fatty acid and oxidation parameters. In the present study, we observed a different fatty acid distribution between patients and control subjects. VLDL and LDL, isolated from HTG patients, contained a higher absolute amount of SFA and a lower relative amount of PUFA than did the corresponding lipoprotein fractions of control subjects, possibly explaining the higher resistance of VLDL and LDL to oxidation in HTG patients.

Differences in the dietary fatty acid composition cannot explain our data, because the HTG group was advised to increase the intake of PUFA at the expense of SFA as first-line therapy. Therefore, a higher intake of PUFA would be expected in the HTG group compared with the population-based control subjects. There are some indications that may explain these differences. First, hepatocytes synthesize preferably simple SFAs over more complex unsaturated fatty acids. Accordingly, an increased supply of substrates to the liver as encountered in HTG may lead to a higher incorporation of SFA compared with PUFA in triglycerides. Second, PUFAs decrease VLDL production, which may cause the liver to incorporate PUFA at a slower rate than SFA. Indeed, a decreased VLDL production has been reported in humans fed a diet rich in PUFA. A third explanation may be preferential lipolysis of triglycerides that are rich in PUFA, as demonstrated by Botham et al. Thus, in HTG patients, hydrolysis of PUFA might be preferred over hydrolysis of SFA. However, on bezafibrate therapy, no change in PUFA content in VLDL was observed, hereby questioning the latter explanation.

The LDL particles in the HTG patients were smaller than those in the control subjects. Unexpectedly, this small dense LDL was associated with an increased resistance to copper-induced oxidation. In addition, on bezafibrate therapy, LDL particle size enlarged, whereas LDL oxidizability increased. These observations are in conflict with reports suggesting that small LDL is particularly prone to oxidation. However, O’Neal et al reported an increase in LDL size without any change in LDL oxidizability on gemfibrozil therapy in patients with type II diabetes, and Makimattila et al showed the occurrence of decreased LDL size along with normal LDL oxidizability in diabetic patients compared with control subjects. These observations suggest that other determinants, such as fatty acid composition and vitamin E content, may be more important in determining LDL oxidizability than LDL size, per se, as discussed above.

There is controversy regarding the effects of fibrate therapy on lipoprotein oxidizability. Some groups have reported an enhanced resistance to oxidative stress whereas others have found no effect. F₂-isoprostane levels have not been studied yet in relation to fibrate therapy. Inasmuch as the present study showed that bezafibrate therapy was associated with normalization of oxidation resistance of isolated lipoproteins and an increase in urinary excretion of F₂-isoprostanes, these observations strongly suggest that bezafibrate therapy increases lipid oxidation in HTG patients. However, the underlying mechanism for bezafibrate-induced enhanced oxidation is unclear. The bezafibrate-induced decrease in the number of VLDL double bonds correlated significantly with the decrease in VLDL lag time. However, only a minor reduction in VLDL double bonds on bezafibrate therapy was observed, suggesting that other factors are involved in the bezafibrate-induced enhanced oxidation. One of these factors may be elevated levels of fasting homocysteine, which are associated with enhanced in vivo lipid peroxidation as measured by iPF₂. We observed, in accordance with a study of Dierkes et al, an increase in serum homocysteine on bezafibrate therapy, which might contribute to the enhanced oxidation of lipoproteins.

In conclusion, we have found normal urinary levels of 2 F₂-isoprostanes and an enhanced resistance of VLDL and LDL to in vitro oxidation in HTG patients, indicating that HTG is not associated with enhanced oxidative stress. The enhanced resistance of HTG lipoproteins to copper-induced oxidation may be explained by a low ratio of PUFA to SFA in VLDL and LDL of HTG patients. Bezafibrate therapy resulted in an increase in F₂-isoprostanes and in normalization of the oxidation resistance of HTG lipoproteins.

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References


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