Gemfibrozil Reduces Small Low-Density Lipoprotein More in Normolipemic Subjects Classified as Low-Density Lipoprotein Pattern B Compared With Pattern A

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We tested the hypothesis that gemfibrozil has a differential effect on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) subclass distributions and postprandial lipemia that is different in subjects classified as having LDL subclass pattern A or LDL pattern B who do not have a classic lipid disorder. Forty-three normolipemic subjects were randomized to gemfibrozil (1,200 mg/day) or placebo for 12 weeks. Lipids and lipoproteins were determined by enzymatic methods. The mass concentrations of lipoproteins in plasma were determined by analytic ultracentrifugation and included the S_f intervals: 20 to 400 (very LDL), 12 to 20 (intermediate-density lipoprotein), 0 to 12 (LDL), and HDL_2 mass ($F_{1.20}$ 3.5 to 9.0) and HDL_3 mass ($F_{1.20}$ 0 to 3.5). Postprandial measurements of triglycerides and lipoprotein(a) were taken after the patients consumed a 500 kcal/M^2 test meal. Treatment with gemfibrozil, compared with placebo, significantly reduced fasting plasma triglycerides (difference from placebo ± SE; −50.2 ± 20.6 mg/dl, p = 0.02), total cholesterol (−16.4 ± 7.5 mg/dl, p = 0.04), apolipoprotein B (−16.1 ± 5.5 mg/dl, p = 0.006), very LDL mass of S_f 20 to 400 (−50.8 ± 24.1 mg/dl, p = 0.02), S_f 20 to 60 (−17.5 ± 8.5 mg/dl, p = 0.05), S_f 60 to 100 (−16.2 ± 8.1 mg/dl, p = 0.05), and increased peak S_F (0.48 ± 0.27 Svedberg, p = 0.08). Gemfibrozil reduced the postprandial triglyceride level significantly at 3 (p = 0.04) and 4 (p = 0.05) hours after the test meal. A significantly different subclass response to gemfibrozil was observed in those with LDL pattern A versus B. Those with LDL pattern B had a significantly greater reduction in the small LDL mass S_f 0 to 7 (p = 0.04), specifically regions S_f 0 to 3 (p = 0.009) and S_f 3 to 5 (p = 0.009). In conclusion, normolipemic subjects with either predominantly dense or buoyant LDL respond differently to gemfibrozil as determined by the changes in LDL subclass distribution. Thus, treatment with gemfibrozil may have additional antiatherogenic effects in those with LDL pattern B by decreasing small dense LDL that is not apparent in those with pattern A. **(Am J Cardiol 2005;96:1266–1272)**

Although subjects classified as having low-density lipoprotein (LDL) subclass pattern A and B have been shown to respond differently to diet and nicotinic acid therapy,[1,2] a differential lipoprotein subclass response to fibrate in normolipemic patients has not been reported. Specifically, we hypothesized that those with LDL pattern B would exhibit significantly greater reductions in fasting plasma concentrations of small, dense LDL and intermediate-density lipoprotein, postprandial lipemia, and a significantly greater increase in high-density lipoprotein $(HDL)_2$ concentrations compared with identically treated subjects with LDL pattern A. We therefore tested, in a randomized, controlled clinical trial, whether subjects with normolipemic LDL patterns A or B responded differently to gemfibrozil treatment. After a meal, a significant change can occur in postprandial lipemia that may affect coronary artery disease risk.[3] Therefore, we also examined whether the postprandial lipemia response to gemfibrozil treatment was related to the LDL subclass pattern.

Methods

Subjects and trial design: In a double-blind, randomized, placebo-controlled trial design, 43 normolipemic sub-

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jects (38 men and 5 women; aged 21 to 65 years) were randomized to gemfibrozil (1,200 mg/day) or placebo for 12 weeks after a 6-week American Heart Association phase I diet wash-out period. Subjects were excluded if they had a medical condition or used medications that could influence plasma lipoproteins. Subjects were required to have total cholesterol values of <250 mg/dl and fasting triglyceride values of <300 mg/dl. All participants completed 4-day diet records at baseline and at the end of treatment. These were analyzed for total calories; percentage of calories from fat, carbohydrate, protein, and alcohol; cholesterol content; polyunsaturated/saturated fat ratio; and micronutrients. The body mass index (BMI) was calculated as kilograms of weight divided by height in meters squared. The institutional human use committee approved the study, and all subjects signed an approved informed consent form.

A modified definition of the metabolic syndrome was used to explore the potential difference in patients with the metabolic syndrome.[4] Because uncontrolled hypertension and medications that affect plasma lipoproteins were exclusion criteria, hypertension was not present in this population. For the purposes of analysis, the metabolic syndrome was defined as fasting triglycerides of >150 mg/dl and a BMI >25 kg/m^2.

Baseline fasting blood samples for lipid and lipoprotein analysis were obtained from the patients after a 12- to 16-hour fast, avoidance of vigorous physical activity, and before the initiation of any therapy. Venous blood samples were drawn with the subject in a sitting position. Postprandial measurements of triglycerides and lipoprotein(a) (Lp(a)) were made after the patients consumed a 500 kcal/M^2 test meal of cream, sugar, and milk that contained 45% of its calories from fat. The test meal was consumed after the fasting blood draw. Additional blood samples were taken at 3, 4, 5, and 8 hours after the test meal while the subjects remained in the clinic area and consumed nothing except water.

Laboratory methods: Plasma was prepared from the blood samples within 30 minutes and the blood and plasma samples were kept at 4°C. Plasma lipid and lipoprotein cholesterol concentrations were determined using the methods of the Lipid Research Clinics.[5] Triglycerides, total cholesterol, and lipoprotein cholesterol values were measured by enzymatic procedures (Abbott ABA 200 instrument, Abbott Park, Illinois). HDL cholesterol was determined by the dextran sulfate-magnesium precipitation procedure.[6] LDL cholesterol was calculated from the following equation: LDL cholesterol = total cholesterol − [HDL cholesterol + (triglycerides/5)]. During the study, the laboratory remained standardized for lipid measurements through the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipoprotein Standardization Program.[7]

The concentrations of lipoproteins in plasma (as total mass) were determined at the Donner Laboratory, University of California at Berkeley, using computer analysis of the results of analytic ultracentrifugation.[8] This technique generates a "Schlieren curve," which describes the distribution of lipoproteins according to their high-density flotation (F) and low- to very-low-density flotation (S_f) rates. The intervals include the total lipoprotein mass concentrations of the following S_f intervals: 20 to 400 (very-low-density lipoprotein [VLDL]), 12 to 20 (intermediate-density lipoprotein), and 0 to 12 (LDL). The HDL_2 mass and HDL_3 mass were determined as the sum of the flotation intervals $F_{1.20}$ 3.5 to 9.0 and $F_{1.20}$ 0 to 3.5, respectively. Classification of LDL pattern A versus B was based on whether the LDL peak (S_F) was ≥5.2 (pattern A) or <5.2 (pattern B).[9]

Apolipoprotein A-I and B assays were performed by a competitive enzyme-linked immunoassay procedure using well-characterized and specific monoclonal antibodies.[10,11] Lp(a) concentrations were measured with an enzyme-linked immunosorbent assay kit (Macra Lp(a) Terumo Diagnostics Division, Tokyo, Japan). Internal quality assurance for apolipoproteins was monitored at 2 levels for each analyte on an on-going basis using specifically prepared frozen pools. Throughout the period in which all apolipoprotein measurements were performed, the laboratory participated in the Centers for Disease Control–International Union of Immunology Societies apolipoprotein standardization program.[12] Apolipoprotein E isoforms were determined by isoelectric focusing of VLDL apolipoproteins and phenotypes designated according to recommended nomenclature.[13]

Statistical analysis: Mean differences between placebo and gemfibrozil were compared using an unpaired *t* test. Two-way analysis of variance was used to test whether treatment differences in the lipoprotein change between baseline and follow-up were affected by the LDL pattern at baseline. Correction for the change in BMI was performed using analysis of covariance.

Results

Baseline values: No significant differences were found between the treatment arms for baseline BMI, lipids, apolipoproteins, Lp(a), VLDL mass distribution, intermediate-density lipoprotein mass distribution, LDL mass distribution, HDL mass distribution, postprandial triglycerides, or Lp(a) (Table 1). The distribution of the apolipoprotein E isoform was not different between groups. Of the patients in the gemfibrozil and placebo groups, 15 and 17 had apolipoprotein E 3/3, 4 and 4 had apolipoprotein E 4/3 or 4/4, and 2 and 1 had apolipoprotein E 2/3, respectively. The treatment arms also did not differ in their nutrient intake at baseline, including dietary cholesterol, total calories, percentage of calories from saturated, unsaturated, and monounsaturated fats, and percentage of calories from carbohydrates and alcohol (analyses not shown). As expected, subjects with pattern B had significantly higher mean triglycerides, VLDL (S_F 20 to 400), intermediate-density lipoprotein (S_F12 to 20), and apolipoprotein B and significantly lower HDL cholesterol, HDL_2 mass, HDL_3 mass, and apolipoprotein A-I concen-