Effects of Fibrinogen on Lipid Peroxidation in Patients with Coronary Heart Disease and *in Vitro*

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In patients with coronary heart disease oxidizability of lipids during Cu^{2+}-induced oxidation of blood plasma inversely correlated with fibrinogen content. A positive correlation was found between the amount of lipid peroxidation products in the plasma from these patients and fibrinogen content. The increase in fibrinogen content was associated with high levels of total lipids and triglycerides and low concentration of high-density lipoprotein cholesterol. *In vitro* experiments demonstrated that fibrinogen reduces oxidizability of blood plasma. Our results suggest that the decrease in lipid oxidizability at high concentration of fibrinogen in patients with coronary heart disease is related to predominant oxidation of fibrinogen and its competition with plasma lipids during Cu^{2+}-induced oxidation.

Key Words: *fibrinogen; lipid peroxidation; blood plasma and serum; coronary heart disease*

Free radical processes of lipid peroxidation (LPO) play a key role in the initiation and development of atherosclerosis and coronary heart disease (CHD). Disturbances in thrombus formation are another risk factor for CHD. The risk of thromboses during cardiovascular disorders is associated with high concentration of fibrinogen (FG) playing a central role in hemostasis. There are contradictory data on the relationship between FG content and intensity of LPO during CHD. A positive correlation was found between the concentrations of malonic dialdehyde (MDA) and FG in middle-aged men [14]. In patients with diabetes mellitus FG content negatively correlates with total antiradical activity of the plasma [8]. However, FG *in vitro* reduces the intensity of low-density lipoprotein oxidation in a cell-free system [10,13]. It was hypothesized that fibrinogen and vitamins C and E act as antioxidants protecting lipoproteins in organs and tissues [10].

In clinical and biochemical assays the intensity of LPO in patients is determined by oxidizability of lipids in the plasma or serum during *in vitro* induction with Cu^{2+} salts. FG is present in the plasma, but not in the serum. It can be hypothesized that the presence of FG in blood plasma modifies lipid oxidizability and affects the results of measurements.

Here we evaluated the relationship between FG content, intensity of LPO, and Cu^{2+}-induced oxidizability of blood plasma in patients with CHD. The effects of exogenous FG on oxidizability of blood plasma and serum were studied *in vitro*.

MATERIALS AND METHODS

We examined 45 CHD patients (functional class II-III). The blood was stabilized with sodium citrate. The plasma was isolated and oxidized on the same day. To

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study oxidizability, blood plasma or serum diluted 10-, 20-, and 50-fold with a buffer containing 150 mM NaCl and 10 mM NaH_2PO4 (pH 7.4) in the presence of 0.05 mM Cu^{2+} at 37°C. The intensity of oxidation was determined by accumulation of conjugated dienes, ketodienes, and MDA.

To study the effect of exogenous FG, the plasma and serum were taken from fasting normolipidemic conventionally healthy volunteers (20-35 years). Exogenous FG was added (2 mg per ml plasma).

The amount of LPO products (conjugated dienes and ketodienes) in lipid extracts of blood plasma and serum was measured spectrophotometrically. Lipid content was estimated gravimetrically [3,4]. MDA concentration was determined in the reaction with thiobarbituric acid [2,15]. The total cholesterol content and concentrations of triglycerides and high-density lipoprotein (HDL) cholesterol were measured on a Centrifichem-400 automatic analyzer using enzyme kits (Boehringer Mannheim GmbH). HDL cholesterol level was estimated after precipitation of apo-B-containing lipoproteins with phosphotungstic acid and $MgCl_2$ [7]. Plasma FG content was measured as described elsewhere [5].

The results were analyzed by methods of variational statistics. The differences were considered to be significant at $p<0.05$.

RESULTS

FG and lipoproteins are synthesized in the liver, belong to acute phase proteins, and determine blood viscosity and platelet aggregation. Normal blood FG content varies from 2 to 4 mg/ml. Previous studies showed that FG more easily undergoes oxidation than other plasma proteins [6,9].

In contrast to blood plasma the serum contains no FG and anticoagulant. To evaluate the effects of these factors on the intensity of Cu^{2+}-induced oxidation we compared oxidizability of blood plasma and serum. The intensity of Cu^{2+}-induced oxidation in the serum was 25-30% higher than in the plasma from the same donor (Fig. 1). These differences were less pronounced after addition of sodium citrate to the serum. The effect of sodium citrate on the intensity of Cu^{2+}-induced oxidation is probably related to the formation of complex compounds with copper. The addition of FG decreased oxidizability of the plasma and serum by 26 and 20%, respectively. Oxidizability of the plasma and serum was nearly similar after addition of FG and sodium citrate. Previous experiments revealed a similar decrease in b-lipoprotein oxidizability during Cu^{2+}-induced oxidation [10]. β-Lipoproteins isolated from the plasma underwent less pronounced oxidation than those obtained from the serum. The plasma contains sodium citrate and FG, whose content markedly changes during cardiovascular disorders. Therefore, the selection of blood plasma or serum during Cu^{2+}-induced LPO determines the results of measurements of oxidizability.

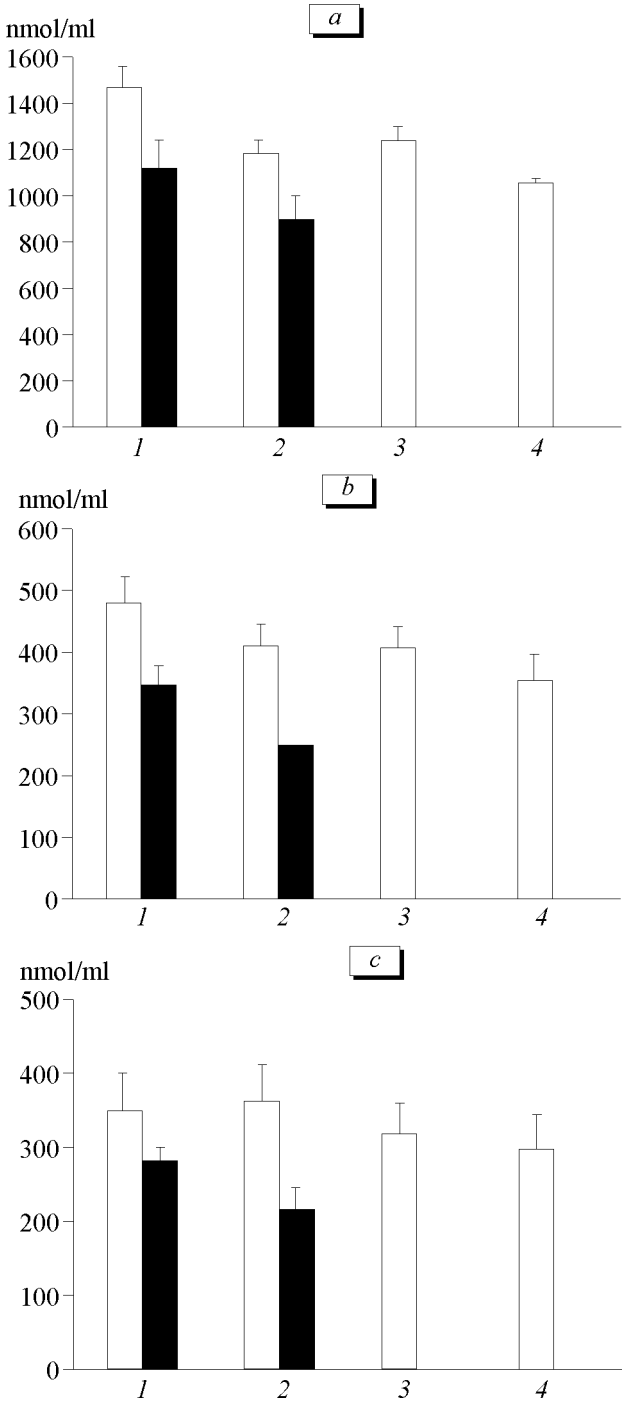

Fig. 1. Effects of fibrinogen and sodium citrate on oxidizability of blood serum (light bars) and plasma (dark bars) in the model system of Cu^{2+}-induced oxidation (6 independent measurements): accumulation of conjugated dienes (*a*), ketodienes (*b*), and MDA (*c*). Samples diluted by 20 times were subjected to oxidation at 37°C for 24 h. No additives (*1*); addition of fibrinogen (*2*), sodium citrate (*3*), and sodium citrate and fibrinogen (*4*).

We compared oxidizability of blood plasma and serum during Cu^{2+}-induced LPO and FG content in patients with CHD. Oxidizability of plasma lipids decreased with the increase in FG content. A negative correlation was found between FG content and concentration of LPO products (Fig. 2). Linear correlation coefficients for conjugated dienes, ketodienes, and MDA were -0.45, -0.48, and -0.44, respectively. Therefore, the increase in FG content in CHD patients and *in vitro* addition of exogenous FG to the plasma were accompanied by a decrease in oxidizability of blood plasma.

FG easily undergoes oxidative modification (*e.g.*, in the presence of Cu^{2+}) [6,9,11,12]. The decrease in oxidizability of blood lipids accompanying the increase in FG content in patients with CHD and under *in vitro* conditions reflects competition between FG and lipids during Cu^{2+}-induced LPO. In healthy donors plasma FG content is comparable with lipid concentration (2-4 and 4-6 mg/ml). In patients with CHD these parameters are 3-8 and 4-8 mg/ml, respectively. Thus, it is incorrect to compare FG with known antioxidants. Antioxidants inhibit oxidation in low concentrations. However, blood FG content little differs from lipid concentration.

We compared FG content with the initial concentration of LPO products in blood plasma measured before oxidation. In CHD patients with high content of FG the amount of LPO products in the blood surpassed that in patients with low concentration of FG. In CHD patients the content of plasma FG positively correlated with the amount of conjugated dienes and ketodienes (correlation coefficients 0.47 and 0.42, respectively, Fig. 3, *a*; data on ketodienes not shown). We studied the relationship between FG content and intensity of lipid metabolism in patients with CHD. The content of FG positively correlated with the concentrations of triglycerides and total lipids (correlation coefficients 0.41 and 0.47, respectively). A negative correlation was found between FG content and HDL cholesterol level (r=-0.47).

These data show that in CHD patients high content of FG the concentrations of LPO products, total

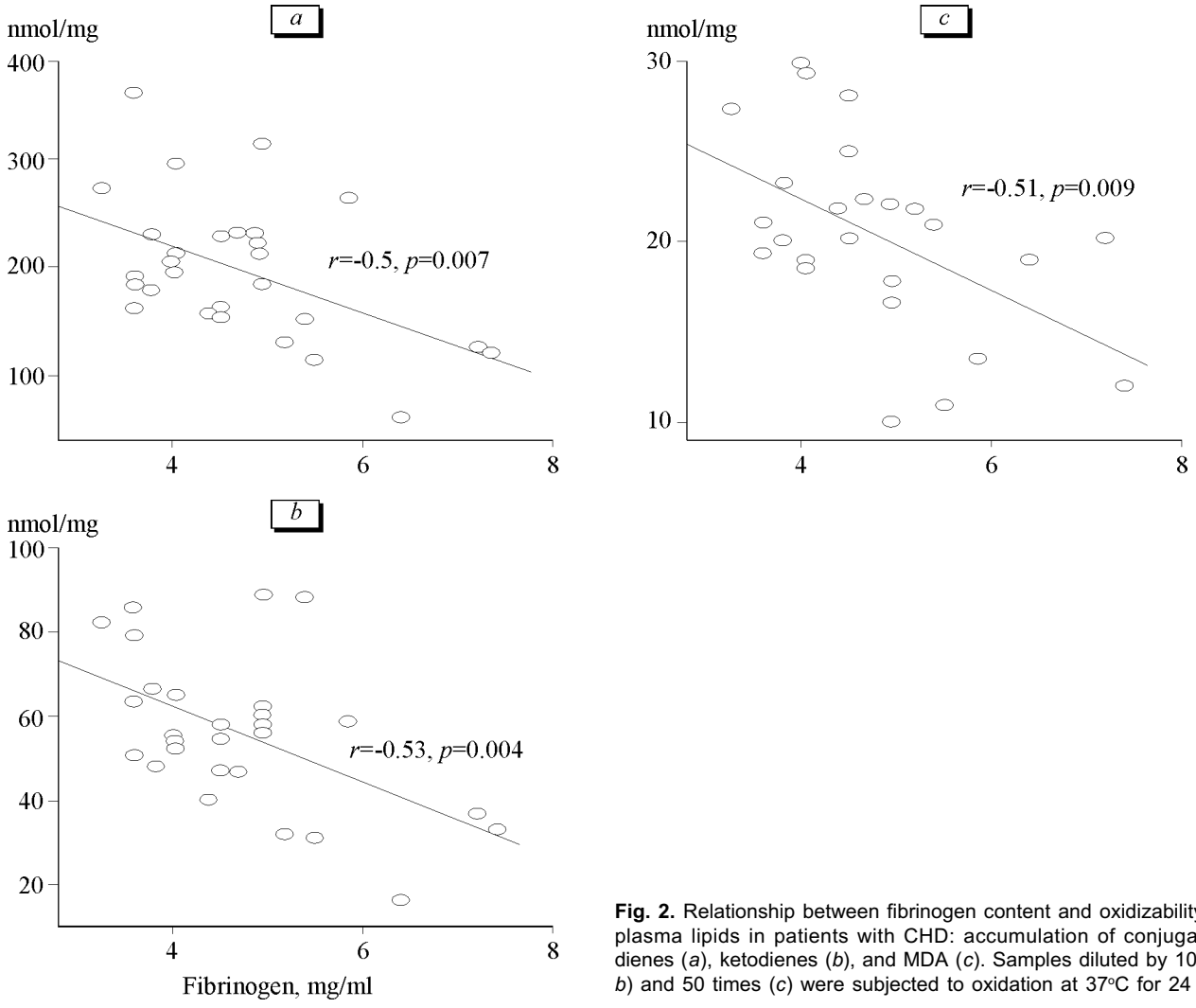

Fig. 2. Relationship between fibrinogen content and oxidizability of plasma lipids in patients with CHD: accumulation of conjugated dienes (*a*), ketodienes (*b*), and MDA (*c*). Samples diluted by 10 (*a*, *b*) and 50 times (*c*) were subjected to oxidation at 37°C for 24 h.

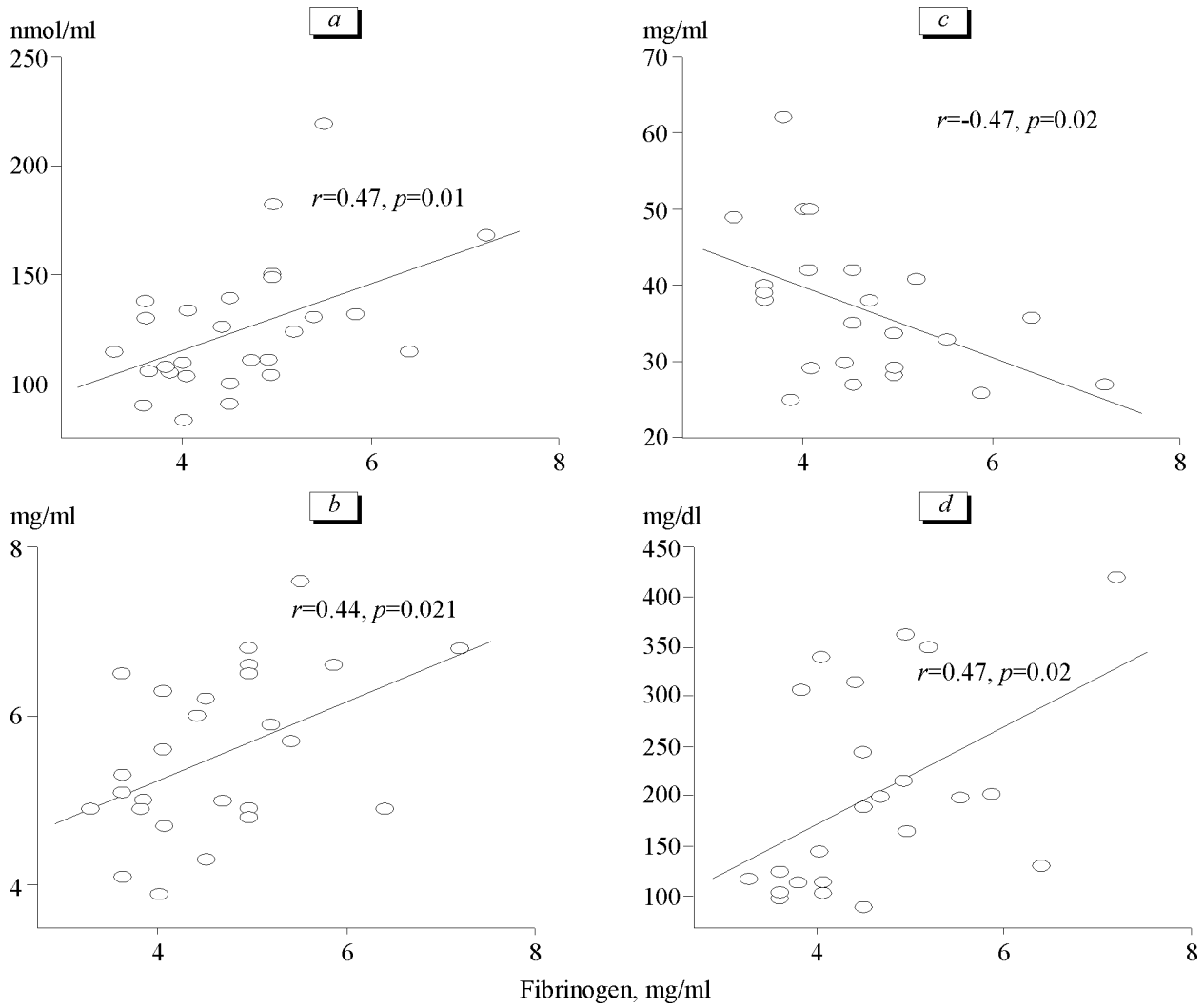

Fig. 3. Relationship between fibrinogen content and concentrations of conjugated dienes (*a*), total lipids (*b*), triglycerides (*c*), and HDL cholesterol (*d*) in the plasma from patients with CHD.

lipids, and triglycerides increse, while the level of HDL cholesterol and oxidizability of plasma lipids decrease due to competition between FG and lipids in the reaction of oxidation. Although high content of FG is associated with low oxidizability of plasma lipids, hyperfibrinogenemia cannot be considered as the defense response that reduces the content of LPO products in the blood from patients with CHD. FG interacts with free radicals and is converted into an oxidatively modified compound. Oxidized FG can produce a direct prothrombotic effect. Published data show that oxidation of FG in the presence of Cu^{2+} is followed by the formation of insoluble polymeric clots [11,12]. Our previous studies showed that oxidation produces changes not only in the structure, but also in functional activity of FG [1]. Oxidized lipids modify the protein molecule of FG. After long-term incubation of samples, highly oxidized lipids suppress fibrinolysis and cause hypercoagulation of the blood.

Our results indicate that although FG reduces oxidizability of blood lipids, its competition with lipids during LPO is a potentially unfavorable factor increasing the risk of thrombosis.

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