Primary Biliary Cirrhosis and Coronary Atherosclerosis: Protective Antioxidant Effect of Bilirubin

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Increased concentration of lipid peroxidation products in patients with primary biliary cirrhosis is related to elevation of serum lipid content, but not to activation of lipid peroxidation. Hyperbilirubinemia in patients with primary biliary cirrhosis is accompanied by a decrease in the concentration of lipid peroxidation products and increase in antioxidant activity of blood serum. Antioxidants play a major role in the prevention of atherosclerosis. We hypothesized that the absence of increased risk of atherosclerosis in patients with primary biliary cirrhosis is due to inhibition of lipid peroxidation in blood serum by antioxidant compound bilirubin.

Key Words: *lipid peroxidation; primary biliary cirrhosis; atherosclerosis; bilirubin; antioxidants*

Increased serum content of lipids, primarily LDL cholesterol (CH) and triglycerides (TG), is one of the major risk factors for atherosclerosis. Free radical lipid peroxidation (LPO) plays a key role in the induction of this disease [2,15]. Oxidized LDL damage the vascular wall, induce the inflammatory response, and promote smooth muscle cell proliferation leading to narrowing of the vessels. Antioxidants decrease LDL oxidizability, thus modulating these processes, which determines their use for the prevention and therapy of vascular atherosclerosis [10].

Changes in lipid metabolism are typical of liver diseases. For example, patients with primary biliary cirrhosis (PBC) are characterized by significant increase in the concentration of total lipids in blood serum and elevation of atherogenic lipid fractions (LDL CH and TG). Moreover, the content of LPO products in the blood of PBC patients also increased, which attests to an important role of LPO in the pathogenesis of this disease [3]. However, high risk of atherosclerosis is not typical of PBC patients despite increased level of atherogenic lipid fractions and LPO products in the blood. This fact is usually explained by changes in LDL CH content and apolipoprotein composition of the serum [4,8,12].

Jaundice is another typical sign of PBC. The level of hyperbilirubinemia during this disorder reaches 350-550 μmol/liter or more. Published data show that bilirubin is a potent antioxidant. The inhibitory effect of bilirubin on LPO is realized via its interaction with free radicals [1].

Here we studied the effect of bilirubin on the concentration of LPO products and antioxidant activity of lipids in the serum from PBC patients. We also evaluated the relationship between LPO parameters and changes in lipid composition of blood serum in patients with PBC and coronary atherosclerosis and healthy donors of the same age group.

MATERIALS AND METHODS

We examined 24 patients with PBC, 85 patients with coronary atherosclerosis (CHD functional classes II-III), and 41 healthy donors of the middle and elderly age groups (40-65 years). Serum samples

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were obtained from PBC patients (G. I. Silonova, Hepatology Center, Riga Medical Institute), CHD patients, and donors of the middle and elderly age groups (S. V. Drinitsina, Institute of Physicochemical Medicine). Informed consent for the use of biological samples was obtained from all participants. The diagnostics and therapy were performed by medical personnel.

Serum lipids were extracted and assayed by the gravimetrical method. Phospholipid concentration was estimated from phosphorus content in lipid extracts. The concentration of LPO products (conjugated dienes and ketodienes) was measured spectrophotometrically. The amount of LDL CH and TG was measured on a Centrifichem-400 automatic analyzer using enzyme kits (Boehringer Mannheim GmbH).

Antioxidant activity of lipids in PBC patients was evaluated from thermal oxidation of oleic acid methyl ester. Antioxidant activity of lipids in CHD patients was evaluated from the kinetics of malonic dialdehyde (MDA) accumulation during Cu^{2+}-induced oxidation of the serum (50-fold dilution). In these studies, antioxidant activity of lipids was inversely proportional to the rate of MDA formation. MDA content was measured in the reaction with thiobarbituric acid. The serum was studied on the day of blood sampling.

The results were analyzed statistically. The differences were significant at $p \leq 0.05$.

RESULTS

Disorders in lipid metabolism play an important role in the pathogenesis of CHD and intrahepatic cholestasis during PBC. The content of total lipids and atherogenic lipid fractions (LDL CH and TG) in PBC patients far surpassed the corresponding values in healthy donors and patients with atherosclerosis (Fig. 1, *a*, *b*, *c*). The total lipid content in PBC patients increased by 79% compared to healthy donors and by 65% compared to patients with atherosclerosis, TG content by 118 and 94% and LDL CH content by 162 and 47%, respectively.

Serum content of conjugated dienes in PBC patients was elevated (Fig. 1, *d*), which agrees with published data [3]. However, oxidability of lipids (amount of conjugated dienes per mg lipids) in PBC patients was much lower than in healthy donors of the same age group. The content of ketodienes in serum lipids from patients decreased to a greater extent (by more than 2 times; Fig. 1, *e*). We hypothesized that the increased concentration of LPO products in PBC patients is related to elevation of serum lipid content, but not to activation of lipid peroxidation. This assumption is supported by published data. For example, the amount of reduced glutathione in the liver does not decrease in PBC patients [7]. Moreover, total antioxidant activity of the serum increases in patients with PBC [11]. Antioxidant drugs silymarin and catergen produced no positive therapeutic effect in PBC patients [5].

The amount of LPO products in blood serum and lipids increased in patients with atherosclerosis. The observed changes reflect LPO activation of LPO in patients of this group (as differentiated from PBC patients).

A strong negative correlation was found between the amount of LPO products and total lipid content in blood samples from PBC patients and healthy donors (Fig. 2, *a*, *b*, *c*). In patients with atherosclerosis, the content of LPO products positively correlated with total lipid content. This probably reflects the release of additional amounts of oxidized lipids from hypoxic or inflammatory foci associated with atherosclerotic damage to vessels or dysregulation of LPO in the organism.

LPO primary concerns phospholipids containing more unsaturated fatty acids than neutral lipids. Changes in antioxidant activity of lipids can be related to variations in lipid composition. Enrichment of lipids with easily oxidized fractions and decrease in the content of hardly oxidized fractions against the background of increased antioxidant concentration constitute the physicochemical system of LPO regulation [6]. Therefore we measured serum content of phospholipids in patients. The increase in the concentration of phospholipids in total lipids from PBC patients and healthy donors was accompanied by elevation of lipid antioxidant activity. The linear correlation coefficients were 0.41 and 0.62, respectively (Fig. 2, *e*, *f*). Hence, the elevation of lipid antioxidant activity and decrease in the concentration of LPO products were accompanied by an increase in the content of easily oxidized phospholipids in PBC patients. A negative correlation between these parameters was found in CHD patients (correlation coefficient -0.36). In contrast to CHD patients, the relationship between changes in lipid oxidation rate, lipid composition, and antioxidant properties of serum lipids was not impaired in PBC patients.

Since bilirubin exhibits antioxidant activity, we hypothesized that inhibition of LPO in the blood of PBC patients is related to hyperbilirubinemia. A positive correlation was found between the concentration of lipid-soluble free bilirubin in blood serum and antioxidant activity of lipids in patients with PBC (R=0.86; Fig. 3, *b*). Total bilirubin concentration negatively correlated with the amount of conjugated dienes and ketodienes (R=-0.68 and R=-

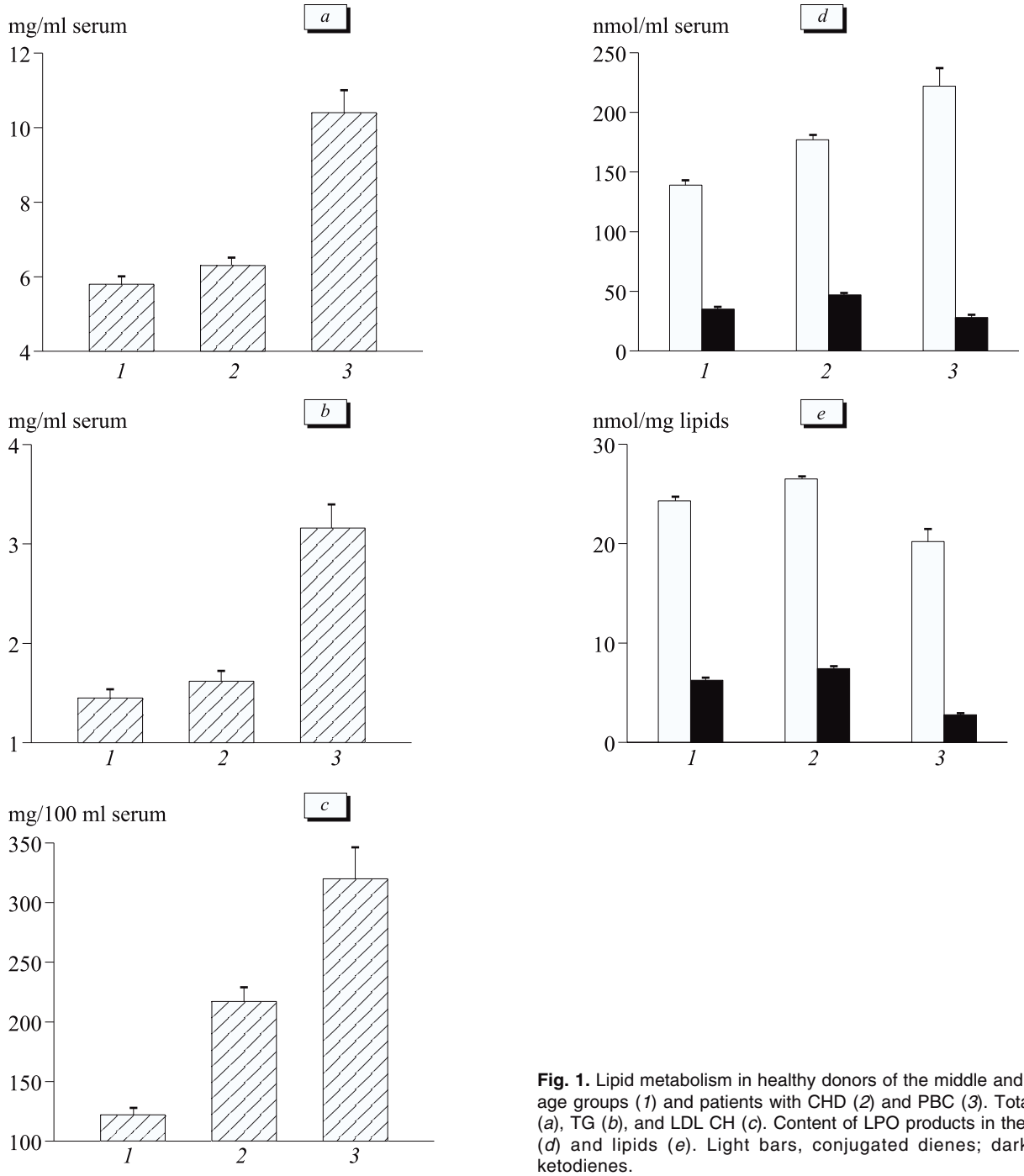

Fig. 1. Lipid metabolism in healthy donors of the middle and elderly age groups (*1*) and patients with CHD (*2*) and PBC (*3*). Total lipids (*a*), TG (*b*), and LDL CH (*c*). Content of LPO products in the serum (*d*) and lipids (*e*). Light bars, conjugated dienes; dark bars, ketodienes.

0.65, respectively; Fig. 3, *a*). Patients with high level of bilirubin in the blood had lower concentration of LPO products and higher content of antioxidants than patients with low level of bilirubin. Our results are consistent with antioxidant properties of this pigment. Previous studies showed that the increase in antioxidant activity of lipids during PBC is determined by antioxidant properties of bilirubin [1].

The protective role of bilirubin in patients with cardiovascular diseases was previously demonstrated [13]. Examination of pilots showed that 50% decrease in blood bilirubin level was associated with 50% increase in the risk of damage to the coronary arteries. Epidemiological studies revealed a direct correlation between blood bilirubin level and amount of HDL CH. However, blood bilirubin level was inversely related to the concentrations of TG, LDL CH, and glucose, systolic pressure, obesity, and cigarette smoking [9,14].

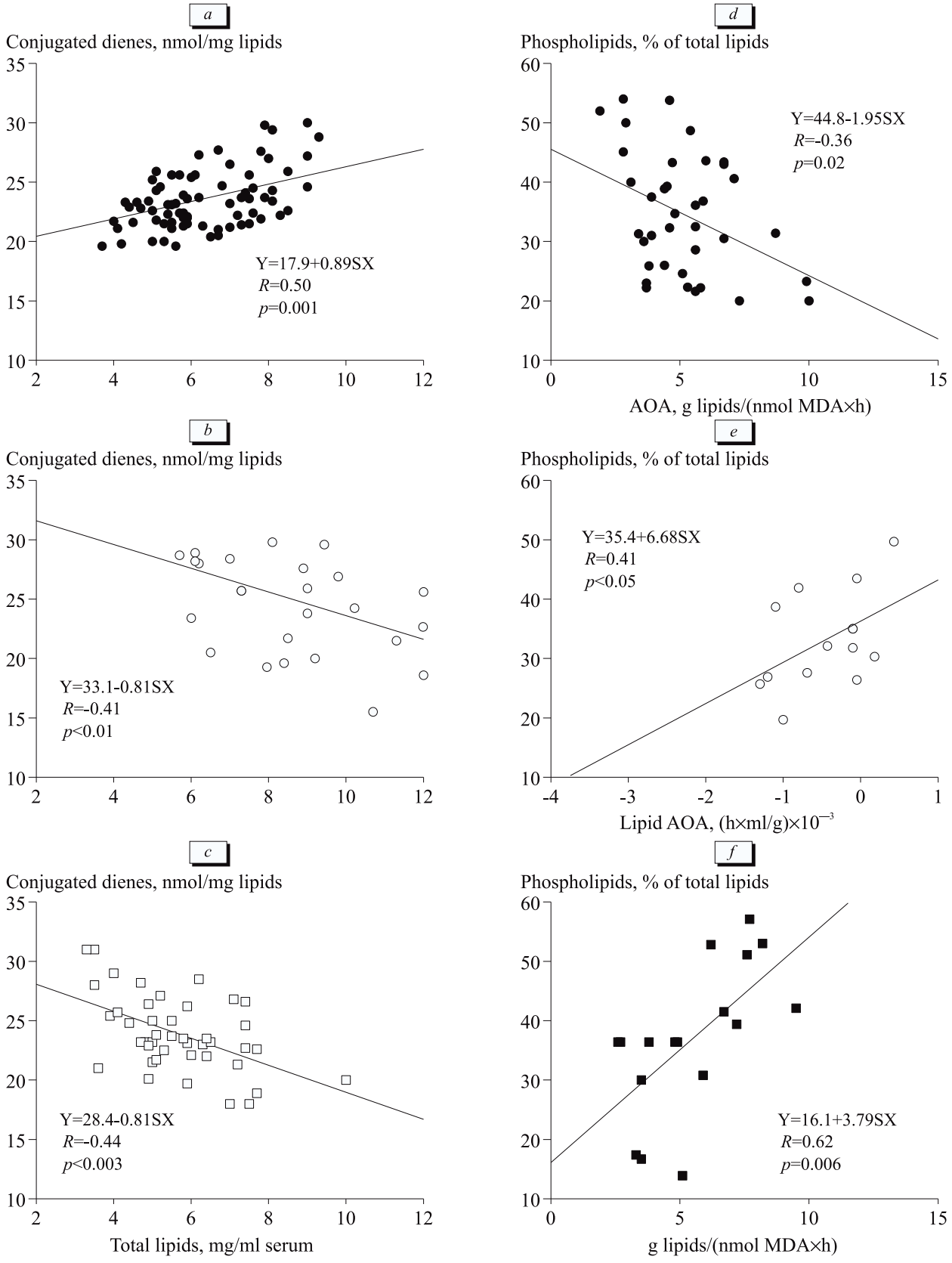

Fig. 2. Relationship between total lipid content and amount of LPO products (*a*, *b*, *c*) and between antioxidant activity of lipids and lipid phospholipid content (*d*, *e*, *f*) in patients with CHD (*a*, *d*) and PBC (*b*, *e*) and healthy donors of the middle and elderly age groups (*c*, *f*). AOA, antioxidant activity.

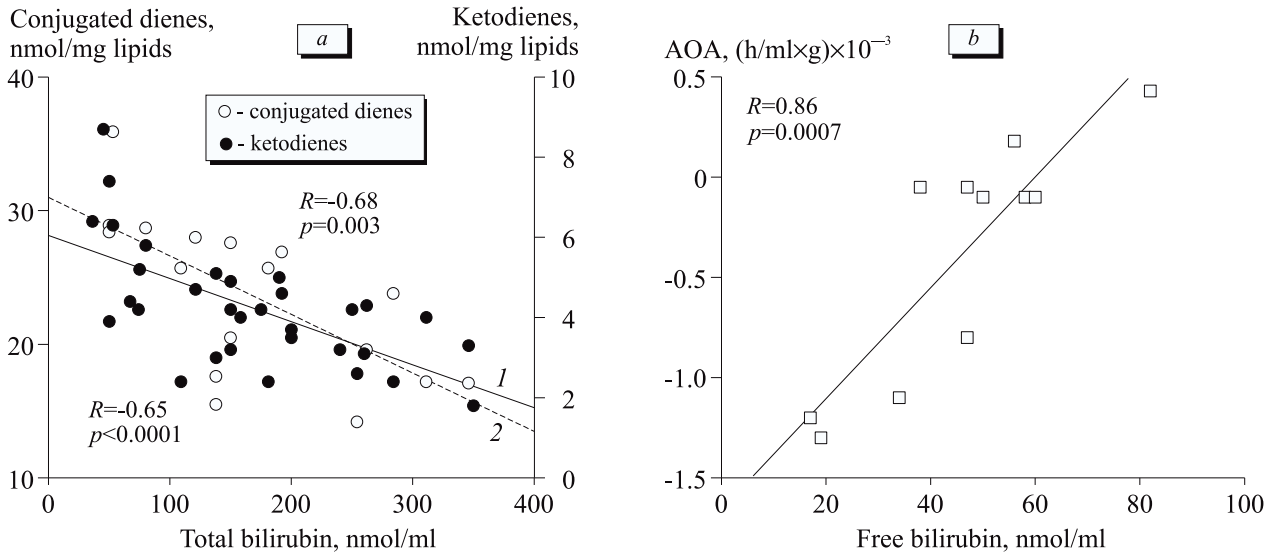

Fig. 3. Relationship of bilirubin level with the amount of LPO products (*a*) and antioxidant activity (AOA) of lipids (*b*) in blood serum from patients with PBC.

We showed that the increased concentration of LPO products in patients with PBC is related to elevation of serum lipid content, but not to LPO activation. The relationship between changes in the content of LPO products in lipids, their composition, and antioxidant properties (*i.e.*, physicochemical system for regulation of oxidation reactions in serum lipids) is not impaired in PBC patients. Potent natural antioxidant bilirubin plays an important role in the regulation of LPO during PBC. Hyperbilirubinemia in PBC patients is accompanied by a significant decrease in the amount of LPO products and increase in antioxidant activity of serum. A strong positive correlation was found between antioxidant activity of lipids and serum bilirubin level in patients. Antioxidants decrease the content of LPO products and play a major role in prevention of atherosclerosis. LPO inhibition in blood serum of PBC patients by antioxidant compound bilirubin probably reduces the risk of atherosclerosis in patients with significant atherogenic changes in the lipid composition of blood serum.

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