Association of Serum Apolipoprotein A-II Concentration with Combined Hyperlipidemia and Impaired Glucose Tolerance

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We studied the relationship of serum apolipoprotein A-II concentration with biochemical parameters of lipid and carbohydrate metabolism, type of hyperlipidemia, and insulin resistance in male patients with hyperlipidemia. High concentration of apolipoprotein A-II was associated with increased indices of atherogenic lipoproteins and high-density lipoprotein-mediated reverse cholesterol transport, combined hyperlipidemia, and decreased insulin resistance calculated with consideration for glucose and insulin levels in glucose tolerance test and body weight.

Key Words: *apolipoprotein A-II; hyperlipidemia; insulin resistance*

Apolipoproteins A-I and A-II (apoA-I and apoA-II, respectively) enter the composition of high-density lipoproteins (HDL). Antiatherogenic function of HDL was shown in epidemiological and clinical trials [11,14]. Protective activity of HDL is related to removal of excess cholesterol (CH) from peripheral tissues, CH transport to the liver, and prevention of oxidative modification of low-density lipoproteins (LDL). Functional activity of HDL is mainly determined by the apoA-I/apoA-II ratio and phospholipid (PL) composition of the particle [5,14]. Antiatherogenic activity of apoA-I was studied in details. However, the role of apoA-II remains unclear. Some authors believe that apoA-II has proatherogenic properties, while others consider it antiatherogenic [4,11,14]. Model experiments with genetically modified mice showed that apoA-II modulates the structure and function and HDL, liver lipase activity, and proteins transporting PL and esterified CH. A positive correlation was revealed between the concentrations of apoA-II, free fatty acids, glucose, and insulin in the plasma [4,6,11,13]. These data indicate that apoA-II has complex metabolic function. It remains unclear whether this function of apoA-II is realized in the human organism. Measurement of apoA-II concentration in patients with various metabolic disorders is of particular importance.

Here we studied the relationship of human serum apoA-II concentration with biochemical parameters of lipid and carbohydrate metabolism, type of hyperlipidemia, and insulin resistance.

MATERIALS AND METHODS

Forty patients (30-49 years) with high levels of total CH, LDL CH, and/or triglycerides (TG) were examined at the State Research Center of Preventive Medicine. The patients had hypercholesterolemia (total CH>190 mg/dl, LDL CH>115 mg/dl, TG <150 mg/dl), hypertriglyceridemia (TG>150 mg/dl, total CH <190 mg/dl), and combined hyperlipidemia (total CH>190 mg/dl, TG>150 mg/dl). The blood was taken from the cubital vein of fasting patients in the morning. The concentrations of glucose, total CH, TG, and HDL CH in the serum were measured after precipitation of apoB-containing

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lipoproteins (apoB-LP) with sodium phosphotungstate in the presence of magnesium chloride. The measurements were performed on an Airone-200 automatic analyzer using Human diagnostic kits. The concentrations of apoA-I, apoA-II, and apoB were estimated by turbidimetric immunoassay using Behring kits. The quality control of biochemical studies was performed according to the requirements of the Federal System of external evaluation of the quality of clinical laboratory tests.

HDL PL concentration was measured in the supernatant after precipitation of serum apoB-lipids. Individual PL were separated by thin-layer chromatography [2]. CH-acceptor activity of serum HDL was estimated by the release of ^{3}H-CH from rat hepatoma Fu5H cells [2]. Oral glucose tolerance test was performed to reveal disturbances in carbohydrate metabolism. Glucose concentration was measured in fasting state (glucose-1) and 2 h after intake of 75 g glucose (glucose-2). The basal (insulin-1) and glucose-stimulated insulin level (insulin-2) was measured by radioimmunoassay (Insuline IRMA, Immunotech). The severity of disturbances in carbohydrate metabolism was estimated according to the criteria of the World Health Organization (1999) [1]. Insulin resistance (HOMA-%S) and functional activity of β-cells (HOMA-%B) were determined in fasting patients using HOMA model software (Homeostatic Model Assessment) [15]. Insulin resistance was also estimated by the insulin sensitivity index ($ISI_{0.120}$). This index was calculated taking into account body weight and concentrations of glucose and insulin before and 120 min after the glucose tolerance test [10].

The results were analyzed by nonparametric tests (Statistica 6.0 software). The data are presented as the median, lower quartile, and upper quartile (median 25-75%). The differences were significant at $p<0.05$.

RESULTS

Hypercholesterolemia in 4 patients was not accompanied by clinical signs of other functional disturbances. Thirty-six hyperlipidemic patients had signs of metabolic syndrome: elevated blood pressure (≥130/85 mm Hg), abdominal obesity (waist circumference≥102 cm), low HDL CH concentration (<40 mg/dl), and hyperglycemia in the fasting state (glucose>110 mg/dl) [8]. Nineteen patients exhibited 3 or more signs of metabolic syndrome. Impaired glucose tolerance was revealed in 13 patients. The tentative diagnosis of diabetes mellitus was made in 7 patients.

Table 1 shows the median values of lipid and carbohydrate parameters in serum samples from patients (terciles of apoA-II concentration). The increase in serum apoA-II concentration in patients of the 1st tercile was associated with higher lipid parameters compared to patients of the 2nd and 3rd terciles (except for HDL CH, glucose-1, glucose-2, and insulin-2). Most patients with combined hyperlipidemia, impaired glucose tolerance, and type II diabetes mellitus belonged to the 3rd tercile. The

TABLE 1. apoA-II Level and Parameters of Lipid and Carbohydrate Metabolism in the Serum of Patients

Parameter	1st tercile (*n*=13)	2nd tercile (*n*=13)	3rd tercile (*n*=14)	*p**
Total CH, mg/dl	223 (207-253)	244 (226-256)	294 (255-322)	0.003
TG, mg/dl	100 (84-179)	134 (100-177)	192 (161-235)	0.113
apoB-LP CH, mg/dl	174 (156-204)	198 (185-212)	237 (205-275)	0.003
HDL CH, mg/dl	46 (37-56)	44 (41-49)	45 (40-50)	0.930
HDL PL, mg/dl	118 (100-134)	125 (104-146)	147 (121-161)	0.087
ApoA-I, mg/dl	112 (106-130)	118 (109-126)	130 (113-142)	0.160
ApoA-II, mg/dl	30.5 (29.7-34.3)	37 (36.0-37.4)	44.5 (42.0-45.5)	0.001
ApoB, mg/dl	101 (93-108)	115 (93-127)	131 (109-146)	0.009
Glucose-1, mg/dl	97 (91-110)	95 (90-108)	122 (107-145)	0.014
Glucose-2, mg/dl	122 (105-147)	111 (105-146)	178 (158-232)	0.019
Insulin-1, μU/ml	15.0 (11.3-26.0)	15.2 (9.0-21.2)	16.4 (12.3-20.0)	0.700
Insulin-2, μU/ml	38.6 (28.6-74.9)	26.0 (22.0-48.1)	62.9 (31.3-98.0)	0.070
HTG/HCS/CHLP	2/9/2	0/7/6	0/3/11	
IGT/DM 2	2/2	2/2	8/4	

Note. *Kruskal-Wallis test. Here and in Table 2: HTG, hypertriglyceridemia; HCS, hypercholesterolemia; CHLP, combined hyperlipidemia; IGT, impaired glucose tolerance; DM 2, type II diabetes mellitus. The upper and lower quartiles are shown in brackets.

TABLE 2. Parameters of Lipid and Carbohydrate Metabolism in the Serum of Patients Depending on IGT

Parameter	IGT^+ (n=20)	IGT^- (n=20)	p^*
Total CH, mg/dl	263 (216-309)	246 (226-256)	0.57
TG, mg/dl	161 (85-214)	148 (97-203)	0.9
HDL CH, mg/dl	45 (39-52)	43 (37-48)	0.34
HDL PL, mg/dl	139 (109-158)	122 (97-140)	0.15
Phosphatidylcholine, %	75 (69-78)	68 (64-72)	0.003
Lysophosphatidylcholine, %	9.7 (9.0-12.8)	14.3 (13.1-18.1)	0.0003
ApoA-II, mg/dl	41.0 (35.8-45.0)	35.5 (32.4-37.1)	0.01
Glucose-1, mg/dl	126.5 (108.5-162.5)	93.5 (87.0-97.0)	0.00001
Glucose-2, mg/dl	182.0 (160.5-245.5)	108.5 (103.5-115.0)	0.00001
Insulin-1, μU/ml	15.0 (12.2-22.8)	15.2 (10.2-22.0)	0.63
Insulin-2, μU/ml	45.2 (26.0-74.0)	36.1 (22.6-74.0)	0.66
HOMA-%S	45.1 (27.6-59.9)	50.9 (36.0-77.7)	0.23
HOMA-%B	66.9 (47.2-119.6)	132.9 (119.8-160.7)	0.001
$ISI_{0.120}$	39.6 (31.7-50.4)	76.2 (62.3-86.3)	0.00001
HTG/HCS/CHLP	2/9/9	0/11/9	

Note. *Mann—Whitney test.

1st and 2nd terciles mainly included patients with hypercholesterolemia and normal carbohydrate metabolism (Table 1). These data illustrate that the increase in blood apoA-II concentration in male patients is associated with combined hyperlipidemia and disturbances in carbohydrate metabolism.

The Spearman rank correlation test was used to evaluate the relationship of apoA-II concentration with lipid and carbohydrate parameters. apoA-II level positively correlated with the concentrations of apoB-LP CH (R=0.59, p<0.0001), TG (R=0.32, p<0.04), apoB (R=0.49, p<0.002), apoA-I (R=0.34, p<0.03), HDL PL (R=0.46, p<0.003), glucose-1 (R=0.31, p<0.05), and glucose-2 (R=0.38, p<0.02). We calculated HDL PL/HDL CH and HDL CH/apoA-I ratios. Increasing the concentration of apoA-II in blood serum was accompanied by a relative increase in PL content in HDL (R=0.42, p<0.01). However, the relative concentration of CH in these particles decreased under these conditions (R=-0.34, p<0.04).

Evaluation of the ability of plasma samples from patients to accept free CH from cell membranes showed that relative CH efflux from Fu5AH cells positively correlated with the concentrations of apoA-II (R=0.46, p=0.003), HDL CH (R=0.37, p=0.02), HDL PL (R=0.58, p<0.0001), and apoA-I (R=0.39, p=0.013). Therefore, the decrease in the relative concentration of HDL CH in serum samples with high level of apoA-II was not related to impairment of CH-acceptor function. This function involves SR-B1 scavenger receptor that is strongly expressed by Fu5AH cells [9].

Published data show that in patients with impaired glucose tolerance HDL are enriched with PL, but depleted of CH [12]. We found that the increase in apoA-II concentration is associated with impaired glucose tolerance. A positive correlation was found between the concentrations of HDL PL and apoA-II in the serum. Probably, the relationship exists between HDL PL concentration and impaired glucose tolerance. Further study involved 2 groups of patients with impaired (IGT^+) or normal glucose tolerance (IGT^-). No intergroup differences were revealed in lipid and protein parameters (Table 2). However, IGT^+ patients were characterized by a higher percentage of HDL phosphatidylcholine and lower percentage of HDL lysophosphatidylcholine. We found no intergroup differences in HOMA-%S. HOMA-%B and $ISI_{0.120}$ in IGT^+ patients were much lower than in IGT^- patients. Independently on glucose tolerance, apoA-II concentration in patients with combined hyperlipidemia was higher than in patients with hypercholesterolemia (p=0.01). Serum apoA-II concentration in patients with disturbances in carbohydrate metabolism was higher than in patients with normal glucose tolerance (p=0.003 and p=0.02, respectively). A negative correlation was revealed between $ISI_{0.120}$ and serum apoA-II concentration (R=-0.41, p=0.009). However, we found no correlation between $ISI_{0.120}$ and HOMA-%S (R=0.04, p=0.81). These data suggest that the increased concentration of apoA-II in patients with hyperlipidemia and impaired glucose tolerance is associated with not only combined hyperlipidemia, but also

hypofunction of β-cells and low insulin sensitivity in the glucose tolerance test. Our results are consistent with published data that apoA-II concentration positively correlates with combined hyperlipidemia [3] and changes in insulin secretion [7].

We conclude that high concentration of apolipoprotein A-II in male patients with hyperlipidemia is associated with the increase in atherogenic lipoprotein parameters (apoB-LP CH, apoB, and TG) and HDL-mediated reverse cholesterol transport (apoA-I and HDL PL) and presence of combined hyperlipidemia. These changes are also associated with disturbances in carbohydrate metabolism. They are manifested in hypofunction of β-cells, impaired insulin resistance, and changes in HDL PL composition. The concentrations of glucose and insulin (glucose tolerance test) and body weight were taken into account to estimate insulin resistance. It can be hypothesized that function of apoA-II in humans is associated with disturbances in lipid and glucose metabolism.

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