ALTERATIONS IN PLASMA LEVELS OF APOLIPOPROTEIN A-IV IN VARIOUS CLINICAL ENTITIES

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Summary

The serum levels of apolipoprotein A-IV (apo A-IV) were measured by rocket immunoelectrophoresis in disease-free humans, at fasting and after oral and intravenous fat administration. The studies were extended to patients with chronic pancreatitis, malabsorption syndrome, to postoperative patients on total parenteral nutrition and to patients with liver diseases, cholestasis, diabetes mellitus and chronic renal failure. Oral fat ingestion resulted in an increase of apo A-IV levels which remained elevated even when the postprandial hypertriglyceridemia had disappeared. A transient increase in apo A-IV levels was observed after intravenous fat infusion but the level declined simultaneously with decreases in triglyceride levels. Levels of serum apo A-IV were decreased under conditions where decreased fat intake or malabsorption of nutrients might have been present, such as in patients with chronic pancreatitis, malabsorption syndrome, acute hepatitis in the early stage, obstructive jaundice and in postoperative patients on total parenteral nutrition. On the other hand, the apo A-IV levels were high in patients with chronic renal failure and in those with diabetes mellitus and proteinuria.

Key Words: Apolipoprotein A-IV, Fat intake, Absorption.

Introduction

Human apolipoprotein A-IV (apo A-IV) is synthesized in the intestine and secreted into the mesenteric lymph as nascent chylomicrons and dissociates from the surface of lymph chylomicrons following entry into the plasma. With ultracentrifugation, apo A-IV is recovered mainly in d>1.21 g/ml fractions of the serum. The metabolic function of this apolipoprotein has not been established. Green and co-workers noted that intestinal synthesis and plasma concentration of apo-IV are increased during lipid absorption. We observed that plasma levels of apo A-IV were decreased in patients with chronic pancreatitis and malabsorption syndrome and suggested that measurements of this apolipoprotein may provide a good index for the assessment of fat intake and absorption.

We extended our work to measure plasma levels of apo A-IV under various pathological states for further elucidation of control mechanism of plasma apo A-IV levels. We also investigated the effects of oral and intravenous
Material and Methods

Fasting serum specimens were obtained from 50 normolipidemic healthy volunteers (26 men and 24 women, aged 18–60), 29 patients with chronic pancreatitis (21 men and 8 women, aged 23–71, 23 alcoholic, 6 idiopathic), 7 patients with malabsorption syndrome (4 men and 3 women, aged 21–67, 2 postgastrectomy, 2 Crohn’s disease, 2 amyloidosis, 1 massive resection of small intestine), 7 postoperative patients during a period of total parenteral nutrition (all men, aged 49–79, 3 gastric cancer, 2 colon cancer, 1 lymphoma of small intestine, 1 gallbladder stone), 117 patients with liver diseases and cholestasis (74 men and 43 women, aged 20–75, 26 acute hepatitis in early stage, 25 chronic hepatitis, 15 compensated liver cirrhosis, 13 decompensated liver cirrhosis, 15 hepatocellular carcinoma, 10 primary biliary cirrhosis, 13 obstructive jaundice), 94 patients with diabetes mellitus (41 men and 53 women, aged 31–81) and 13 patients with chronic renal failure maintained on hemodialysis (5 men and 8 women, aged 35–83). All patients were Japanese.

The diagnosis of chronic pancreatitis was established by an abnormal pancreozymin-secretin test and/or by abnormal findings of the pancreatic ducts by endoscopic retrograde cannulation of the papilla (ERCP). Malabsorption syndrome was diagnosed by indigestion revealed by digestive and absorptive tests (fat balance study and/or D-xylose absorption test and/or vitamin B₁₂ absorption test). The diagnosis of liver diseases was established by usual clinical and biochemical tests and by liver biopsy, in most patients. Obstructive jaundice was diagnosed by clinical findings and by evidence of bile duct obstruction revealed by ultrasound, computerized tomography and/or ERCP. Diabetes mellitus was diagnosed by abnormal glucose tolerance. The patients were divided into two groups, those with and without proteinuria.

Oral and intravenous fat load studies.

After a 12 hour fast, 6 normolipidemic healthy volunteers (3 men and 3 women, aged 24–28) ate 60 g of butter. Blood was sampled by venopuncture before and 1, 2, 3, 4, 6 and 8 hours after this fat intake. Another six normolipidemic healthy males were given 200 ml of Intralipid (a 10% fat emulsion for intravenous use which also contains 1.2% of lecithin and 2.5% of glycerol as an emulsifier), intravenously, during a period of one hour. Blood samples were obtained before and 1, 2, 3 and 4 hours after the start of fat infusion.

Apo A-IV assay.

Isolation of apo A-IV and preparation of antisera. Apo A-IV was isolated from d>1.21 g/ml fractions of combined serum of 4 normolipidemic volunteers, prepared by ultracentrifugation at 40,000 rpm for 24 h. To this material a quarter volume of Intralipid was added and the preparation was mixed well and incubated at 37°C for 1 h. The lipid particles which adsorbed apo A-IV and other proteins were recovered by ultracentrifugation at 30,000 rpm for 16 h after adjusting the density to 1.019 g/ml with NaCl. The lipid particles were treated with ethanol-ether (3/1, v/v) to remove lipids. The delipidated protein was dissolved in 0.1% SDS in 0.01 M Tris-HCl and applied to preparative SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The apo A-IV band in SDS-PAGE was cut out after visualization with 4 M sodium acetate. Apo A-IV was eluted with 0.01 M Tris-HCl buffer containing 0.1% SDS at pH 8.2. The purity of the isolated apo A-IV was checked by SDS-PAGE. Antisera were prepared by giving New Zealand white rabbits 0.5 mg of apo A-IV with an equal volume of Freund’s complete adjuvant. The antisera were tested for specificity by Ouchter-