Symposium on the Disturbance of the Lipid Metabolism in Clinical Field*

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(1) Metabolism of HDL and Its Disorder

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Many investigators reported significant negative correlation between the incidence of coronary artery disease (CAD) and plasma HDL cholesterol (HDL-chol) concentration. Barr, Russ and Eder first realized the intimate association of plasma HDL level and the risk of CAD. Later Glueck et al reported significantly low incidence of CAD in affected subjects from families with familial hyperalphalipoproteinemia. Meanwhile, several pathological and physiological conditions have been disclosed which affect HDL-chol esterol. They include premenopausal females, subjects who engage in strenuous exercise, chronic alcoholism and insulin treatment. In this report we describe the findings obtained from the studies of diabetic subjects and from the in vitro experiments using plasma lipoproteins.

MATERIALS AND METHODS

The subjects of the present study include diabetics, gouts and normal individuals. Major lipoproteins and, HDL₂ and HDL₃ subfractions were separated by ultracentrifugation. Each fraction was dialyzed against physiological saline and if neces-

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sary, concentrated through Diaflo membrane. Total cholesterol, unesterified cholesterol and triglycerides were determined by standard enzyme assays. LCAT activities were determined by using heat-inactivated plasma and LCAT was partially purified from normal human plasma by DEAE cellulose, ammonium sulfate precipitation, hydroxylapatite chromatography and HDL-Sepharose affinity chromatography. HDL was delipidated with chloroform: methanol (2/1, v/v) and the apoproteins were separated into apoA-I and other apoproteins by DEAE cellulose and Sephadex G150 chromatography in the presence of 8M urea. Anti apoA-I sera were obtained from a rabbit immunized with apoA-I. ApoA-I contents present in lipoproteins were determined by immunoelectrophoresis.

RESULTS AND DISCUSSION

1) Vascular complications and HDL-chol. As there exist substantial effects of aging and sex on HDL-chol, 50-65 years old, female diabetics were selected and studied. Among 125 such patients, 13 cases (group A) belonged to hyper- and 18 cases (group B) to hypo-HDL-chol groups, and the HDL-chol levels were consistently higher than 70mg/dl in the former and they were less than 49mg/dl in the latter for approximately 2 years' follow-up period. The mean HDL-chol were 81.6±11.7 mg/dl and 42.5±6.7 mg/dl for group A and B, respectively. Significantly elevated values in group B were; serum triglycerides (182.8 vs 78.8 mg/dl), relative body weight (117 vs 101 %), and systolic blood pressure (147 vs 126 mgHg). However, the items such as serum total cholesterol, fasting blood glucose and diastolic blood pressure, were not different significantly between the two groups. The mean age was 56±4 years for group A and 57±5 years for group B. Aortic calcification was found in 50% in group B, whereas in only 8% it was positive for group A and this difference was significant (p<0.01). The incidence of retinopathy was slightly higher in group B and the difference was weakly significant (p<0.05) between the two groups.

2) The effect of exercise on HDL chol. 35 normal subjects were divided into 5 groups and sex ratio was almost equal in each group. For 20 days they performed rope skipping for 4 minutes with one minute pause in every day. As an increase in the severity of physical exercise, the increase of HDL-chol in the groups with 180 rounds per minute and 220 rounds per minute, revealed a significant increase of HDL-chol as compared with the pre-exercise values.

3) HDL-chol in gout. HDL-chol values were measured in 43 patients with gout. The HDL-chol levels were significantly decreased compared with controls with same triglyceride levels.

4) HDL subfractions in diabetics. HDL subfractions...HDL2 and HDL3 were separated by ultracentrifugation and the cholesterol, phospholipid and apoA-I were determined after dialysis of the samples against physiological saline. Both HDL-chol and HDL apoA-I were increased in female diabetics as compared with male diabetics and this increase in female was reflected by an increase of apoA-I and cholesterol in HDL2 fraction rather than HDL3 of which cholesterol and apoA-I were almost same between them. Treatment of diabetics with insulin brings about an increase of HDL-chol. However, the details of this effect of insulin have not been clearly understood. HDL-chol was significantly higher in insulin group as compared with oral drug group. Concerning subfractions of HDL, HDL2 cholesterol and HDL2 apoA-I increased in parallel with HDL-chol and HDL apoA-I. There were no significant differences in HDL3 chol and HDL3 apoA-I between insulin and oral drug groups.

5) Changes in triglyceride/esterified cholesterol ratio in HDL. As an increase in plasma triglyceride level, HDL TG/CE ratio increased, however, there were no correlations between plasma triglyceride and HDL triglyceride. A significantly negative correlation (p<0.01) was found between

HDL-chol and HDL TG/CE ratio. Moreover, similar relationships were found between both HDL apo-A and HDL_2_ apoA-I with HDL TG/CE, however there were no correlations between HDL TG/CE and HDL_2_ apoA-I.

6) LCAT and HDL metabolism. LCAT was partially purified from normal human plasma by several steps including DEAE-cellulose, ammonium sulfate precipitation, HDL-Sepharose chromatography and hydroxylapatite chromatography. The preferred substrate for LCAT is HDL. Extensive esterification of cholesterol occurs preferentially on HDL, and only slightly the reaction proceeds on LDL. Apparently no enzyme reaction was found on VLDL. The effect of LCAT reaction on cholesterol exchange was studied between HDL and LDL. For the purpose of easy separation of LDL from the incubation mixtures, this lipoproteins were coupled to CNBr-activated Sepharose. The mixtures containing HDL and LDL-Sepharose were incubated at 37°C for 18 hrs in the presence of active or heat-inactivated LCAT and at the end of incubation, the supernatant HDL and LDL-Sepharose gels were separated by centrifugation. The cholesterol contents of HDL increased significantly and those of LDL-Sepharose decreased significantly when the incubation media contained active LCAT, thus the fact becomes evident that HDL retain the capacity to absorb cholesterol from LDL in concert with LCAT reaction.

In the next series of the experiments, the effect of LCAT reaction on the transfer of apoproteins from HDL was studied. HDL-Sepharose was incubated with or without active LCAT for 16 hrs at 37°C, then the gels were washed extensively with physiological saline. The gels were hydrolyzed and the aminoacid contents were measured. There were no significant changes in aminoacid contents of HDL-Sepharose incubated with active LCAT when they were compared with those of control gels. In the second experiments, VLDL and HDL were incubated with active or inactivate LCAT for 18 hrs at 37°C and HDL were separated by ultracentrifugation. The apo A-I contents in incubated HDL were measured by rocket immunoelectrophoresis. The apoA-I present in control HDL fraction was 172.1±8.8 μg/medium and 154.2±10.3 μg/medium of apoA-I was present in HDL fraction catalyzed by LCAT. The difference of these values was statistically significant (p>0.01). In the final in vitro experiments, the effect of LCAT incubation on the change of the size of HDL was investigated. Two kinds of experiments were performed. In the first experiments, HDL was incubated with red cell ghosts without active LCAT and in the second experiments, LDL, red cell ghosts and active LCAT were incubated at 37°C for 18 hrs. Each incubation mixture was filtered through Sephadex G200 and the total cholesterol contents in the eluates were determined. Compared to the control experiment, gel filtration pattern of HDL which were incubated with active LCAT and red cell ghosts revealed the shift of mean particle size to bigger molecular fractions. And in these bigger molecular fractions, the amount of HDL_2_ (D=1.063-1.125) were larger. The results of these experiments suggest that LCAT reaction may play a role on the conversion of HDL_3_ to HDL_2_ by increasing the lipid contents of the lipoprotein particles.

**SUMMARY**

1. The change of the plasma HDL-chol concentration is accompanied by changes in the structure of HDL, thus, as HDL-chol decreases, HDL TG/CE ratio increases. Also, HDL_2_ chol and HDL_2_ apoA-I decreases in parallel with the decrease of HDL-chol.

2. Compared to HDL_2_ chol, HDL_2_ apoA-I has more significant relationships to sex, obesity, hypertriglyceridemia in the diabetics of the present study.

3. The in vitro experiments suggested that plasma LCAT reaction has a significant role on the changes of HDL structure and
function which may be involved in cholesterol transport from peripheral tissues to liver.

REFERENCES

(Supplementary) Disorders of Lipid Metabolism Assessed by the Changes in the Plasma Concentrations of Apolipoproteins

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Plasma lipoproteins are made up of lipids and several kinds of apoproteins which are known to determine the structure and function of lipoproteins and to regulate lipid metabolism. Thus, it will be of value to elucidate changes in apoprotein concentrations in the sera from patients with disorders of lipid metabolism.

In this study, the concentrations of apoproteins A-I, A-II, B and C-III were determined by the technique of rocket immunoelectrophoresis using the specific antisera for each apoprotein which had been raised in the rabbits. Plasma samples were obtained from fasted normal subjects, hyperlipidemias, diabetics and patients with hepatobiliary disorders. Changes in apoprotein concentrations were also measured in the plasma obtained from the volunteers who had ingested 55g of butter or received an intravenous injection of 1ml/kg body weight of 10% fat emulsion (Intralipid).

The concentration of A-I and A-II decreased in patients with liver dysfunction and cholestasis including extrahepatic biliary obstruction. The decreases were approximately proportional to the degrees of liver dysfunction but profound decrease were observed when cholestasis existed. In the cases of severe liver dysfunction and cholestasis, the decrease in A-II levels were more pronounced than the decrease in A-I thus resulted increase in A-I/A-II ratio. No significant changes were observed in the levels of A-I and A-II in patients with diabetes mellitus and hyperlipidemia type II and type IV.

Concentrations of apo B in hyperlipidemias both type II and type IV were about 160% of the control value. In patients with chronic hepatitis and compensated cirrhosis, the apo B levels remained approximately in the normal range or slightly elevated. Increased apo B levels were also observed in patients with cholestasis.

Concentration of C-III remained in their normal range in most of the diabetic patients. The concentrations were not related to the severity of the diseases or the levels of fasting sugar but correlated roughly to the levels of triglycerides. In hepatobiliary disorders, the C-III levels decreased...