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STUDY ON CHOLESTERYL ESTER TRANSFER ACTIVITY IN CORONARY HEART DISEASE

BY

Yoshinori FUJINUMA, Akira TANAKA and Hidenori MAEZAWA

ABSTRACT

The net cholesterol transfer activity from high density lipoprotein (HDL) to low density lipoprotein (LDL) was determined in the patients with coronary heart disease (CHD) to examine its effect on the pathogenesis of arteriosclerosis. Furthermore, in the CHD patients with high HDL cholesterolemia (more than 60 mg/dl), the HDL particle size was measured by high performance liquid chromatography. A significant cholesteryl ester transfer activity ($P<0.02$) was noted in the CHD patients with low HDL cholesterolemia (less than 60 mg/dl). The rate of cholesteryl ester transfer activity (cholesteryl ester transfer activity/hour) inversely correlated with the serum HDL cholesterol value ($r=-0.483$, $P=0.096$) in the patients with CHD. These results suggest that an increase of CETA caused a low HDL cholesterol value in the CHD patients with low HDL cholesterolemia and it may have the risk of causing CHD. However, an increase of the CETA was not found in the CHD patients with high HDL cholesterolemia compared to the normal subjects, the HDL particle size being significantly greater than that in the normal subjects. In the CHD patients with high HDL cholesterolemia, the large size of HDL may have the risk of causing CHD.

Key words: Cholesteryl ester transfer protein (CETP), High density lipoprotein (HDL), Coronary heart disease (CHD), High performance liquid chromatography (HPLC), Arteriosclerosis.

INTRODUCTION

Various epidemic studies (Miller et al. [1]; Gordon et al. [2]; Castelli et al. [3]) have indicated that serum high density lipoprotein (HDL) cholesterol values inversely correlate to the incidence of coronary heart disease (CHD) and HDL cholesterol has a preventive effect on arteriosclerosis. As a mechanism of these phenomena, reverse cholesterol transport, an action of HDL to extract cholesterol from the peripheral cells and ultimately transport it to the liver, is considered (Mahley [4]).

During the incubation of serum samples, the transport of cholesteryl ester from HDL to other lipoproteins, especially triglyceride-rich lipoproteins, may occur (Nichols et al. [5]). A cholesteryl ester transfer protein (CETP) promotes this reaction. The CETP is a glycoprotein having a molecular weight of 74,000 and exists as a combined form with HDL. It is also found in a lipoprotein-free fraction. Free cholesterol is transformed to cholesteryl ester by the action of lecithin: cholesterol acyltransferase (LCAT) on HDL after being extracted from the peripheral cells (Jarnagin et al. [6]; Hesler

藤沼悦範，田中　明，前沢秀憲：Third Department of Internal Medicine (Chief: Prof. H. MAEZAWA), Faculty of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku)
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et al. [7]; Morton et al. [8]; Groener et al. [9]; Schmitz et al. [10]; Daerr et al. [11]; Rajaram et al. [12]).

The CETP seems to share a part of the reverse cholesterol transport. But there are many obscure points concerning its in vivo actions, especially its relationship to arteriosclerosis. In this study, the cholesterol transfer activity was thus determined by the incubation method (Nichols et al. [5]) using high performance liquid chromatography (HPLC) in the patients with CHD to examine the effect of CETP on arteriosclerosis. Furthermore, in the patients with CHD, the HDL particle size was measured by HPLC to examine its effect on CHD.

Materials and Methods

I. Cholesteryl ester transfer activity from HDL to low density lipoprotein (LDL)

Ten patients with CHD who had a significant stenosis of more than 75% on coronary angiography and 8 normal subjects were enrolled in this study.

In the CHD patients as compared with the normal subjects, the serum triglyceride value was relatively higher but the serum HDL cholesterol value was lower. Among the CHD patients, 4 cases had diabetes mellitus concurrently. All CHD cases had a HDL cholesterol level of 60 mg/dl or less. Furthermore, three other CHD patients with high HDL cholesterolemia (more than 60 mg/dl) were examined for the cholesterol transfer activity (Table 1).

Table 1. Mean Concentrations of Serum Total Cholesterol, Triglyceride, and HDL Cholesterol of Investigated Subjects

	N	TC (mg/dl)	TG (mg/dl)	HDL · C (mg/dl)
Normal control	8	189±42	76±27	63±9
CHD HDL · C<60	10	218±67	141±53*	39±8**
CHD HDL · C>60	3	200±5	117±27	77±11

*p<0.01 **p<0.01 vs. Normal Mean±SD

After overnight fasting, blood sampling was performed. After serum separation, HDL ($1.063<d<1.210$)+lipoprotein deficient serum ($d>1.210$) was obtained by ultracentrifugation. This fraction was mixed with the LDL fraction (1.006 to 1.063) obtained from a normal subject by ultracentrifugation at a ratio of 4 : 1 and incubated at 37°C (Nichols et al. [5]). The amount of the HDL cholesterol was measured by HPLC before and after incubation and its decrement was regarded as the amount of cholesteryl ester transferred from HDL to LDL. A column of G3000SW (TOSOH, Tokyo, Japan) was used at a flow rate of 0.4 ml/min. Determiner TC555 (Kyowa Medix, Tokyo, Japan) was utilized as a chromophoric reagent. Twenty μl of the serum were charged on the column to separate the lipoproteins and continuously measured at 550 nm to depict its elution pattern. The amount of HDL cholesterol was expressed as a percentage of the HDL area against the total area. A linear relationship between the HDL area and the HDL cholesterol level has already been confirmed (Okazaki et al. [13]).

In a preliminary experiment, to decide on the appropriate incubation time, the cholesteryl ester transfer activity from HDL to LDL was measured at various incubation times. It reached a stationary state after 9 or 12 hours of incubation. Thus, incubation was routinely performed for 18 and 36 hours but for 20 hours in the CHD patients with high HDL cholesterolemia.

The cholesteryl ester transfer between HDL and LDL occurred in both directions and the net cholesterol transport from

HDL to LDL was determined in the present study.

II. HDL particle size in CHD patients with high HDL cholesterolemia

Four patients with angina pectoris or myocardial infarction having a stenosis of more than 75% on coronary angiography despite the high HDL cholesterolemia (more than 60 mg/dl) and 18 normal subjects having high HDL cholesterolemia (more than 60 mg/dl) were examined in this experiment (Table 2). There was no case complicated with diabetes mellitus (Table 3). The apoprotein level showed no abnormalities except for a slightly high apo A–I (K.M.) and apo E (T.N.) (Table 4).

HDL particle size was expressed as an elution time of HDL peak on the elution pattern after the separation of the lipoproteins and successive measurements of triglyceride and cholesterol through an enzymatic reaction (Okazaki et al. [13]; Hara et al. [14]). The particle size becomes smaller with delayed elution times. A column of G5000PW (TOSOH, Tokyo, Japan) was used at a flow rate of 0.4 ml/min. Determiners TG and TC 555 (Kyowa Medix, Tokyo, Japan) were used as reagents for measuring the triglyceride and cholesterol.

Table 2. Mean Concentrations of Serum Total, Cholesterol, Triglyceride, and HDL Cholesterol of Investigated Subjects

	N	TC (mg/dl)	TG (mg/dl)	HDL · C (mg/dl)
Normal control	18	178±19	68±20	79±9
CHD	4	192±13	103±33	77±7

Mean±SD

Table 3. Characteristics of CHD Patients With Hyper-HDL-Cholesterolemia

	Sex	Age (Years)	Tobacco	BP	Obesity Index	Diagnosis
A.I	M	58	20	130/80	116	AP
K.M	M	64	20	110/60	101	MI
T.S	F	73	0	140/60	107	AP
T.N	F	86	0	150/80	103	MI

Table 4. Serum Total Cholesterol, Triglyceride, HDL Cholesterol, LDL Cholesterol, and Apoproteins of CHD Patients With Hyper-HDL-Cholesterolemia

	TC (mg/dl)	TG (mg/dl)	HDL · C (mg/dl)	LDL · C (mg/dl)	Apo A-I (mg/dl)	Apo B (mg/dl)	Apo E (mg/dl)
A.I	206	154	62	113	162	93	4.9
K.M	195	105	81	93	188	79	3.4
T.S	198	91	87	93	178	66	4.4
T.N	170	62	76	82	159	71	7.3

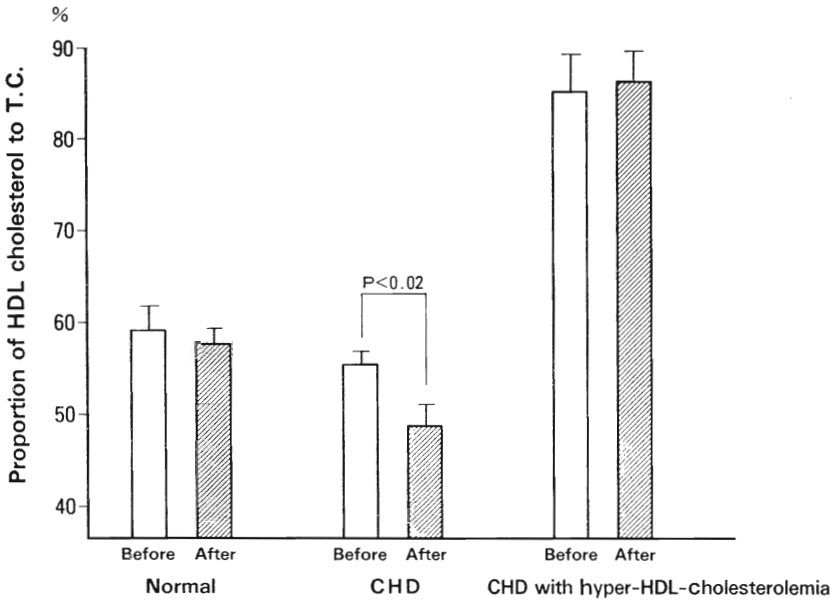


Fig. 1. HDL Cholesterol Content Before and After Incubation.

RESULTS

I. Cholesteryl ester transfer activity from HDL to LDL

The HDL cholesterol levels were compared between before and after incubation. A decreasing trend was observed in the normal subjects, whereas a significant decrease ($P<0.02$) was noted in the CHD patients with low HDL cholesterolemia (less than 60 mg/dl). In the CHD patients with high HDL cholesterolemia, no change was shown before and after incubation showing no evidence of cholesteryl ester transfer activity (Fig. I). The rate of cholesteryl ester transfer activity was calculated by subtracting the cholesteryl ester transfer activity by the time of incubation (%/h). A positive correlation between the cholesteryl ester transfer activity rate and serum HDL cholesterol value was observed in the normal subjects ($r=0.876$) (Fig. 2). On the other hand, a negative correlation was observed in the CHD patients ($r=-0.483$) (Fig 3).

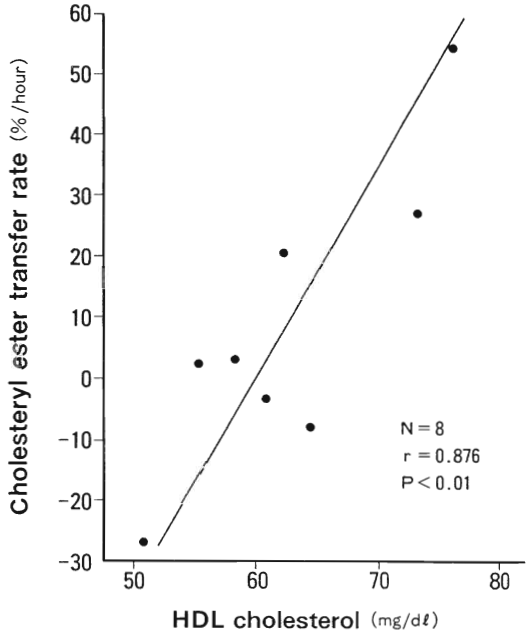
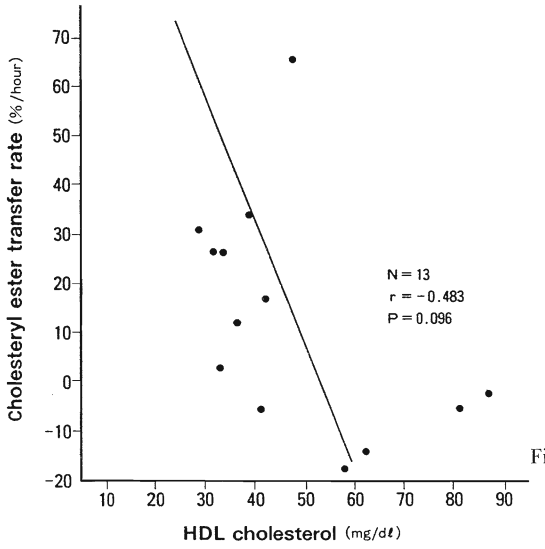


Fig. 2. Correlation Between Cholesteryl Ester Transfer Activity Rate and Serum HDL Cholesterol Value in Normal Subjects.

II. HDL particle size in CHD patients with high HDL cholesterolemia

Triglyceride monitoring of HPLC revealed an elution time of HDL peak being



52.28±0.15 mins (Mean±SD) for the CHD patients and 52.90±0.30 mins for the normal subjects. Thus, it was significantly faster ($P<0.001$) in the CHD patients than in the normal subjects (Fig. 4). In cholesterol monitoring of HPLC, the elution time was significantly faster in the CHD patients (52.85±0.15 mins) than in the normal subjects (53.51±0.23) ($P<0.0001$) (Fig. 5). Thus, the HDL particle size was greater in the CHD patients than in the normal subjects.

Fig. 3. Correlation Between Cholesteryl Ester Transfer Activity Rate and Serum HDL Cholesterol Value in Subjects With Coronary Heart Disease.

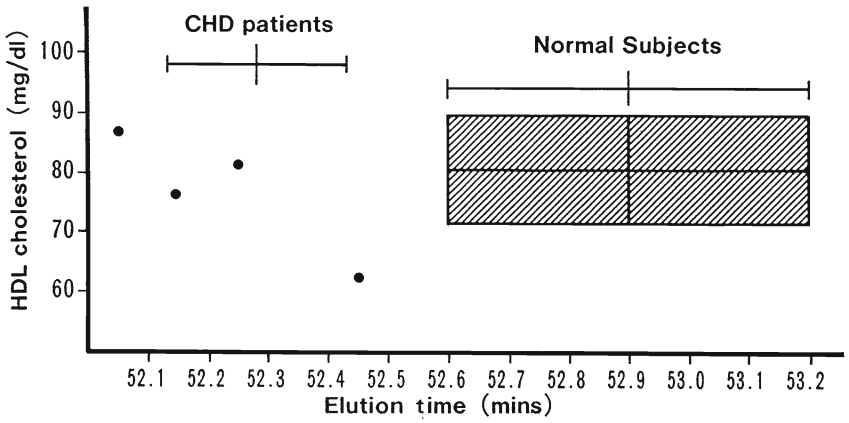


Fig. 4. Comparison of Triglyceride Monitored HDL Particle Size Between CHD With High HDL-cholesterolemia and Normal Subjects With High HDL-cholesterolemia.

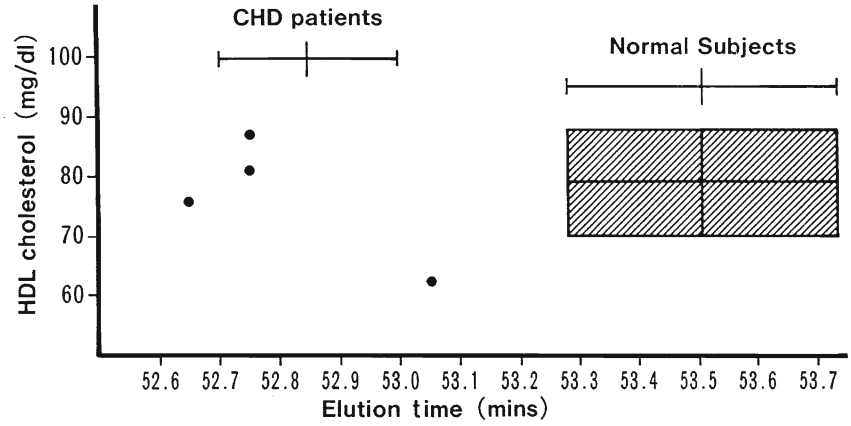


Fig. 5. Comparison of Cholesterol Monitored HDL Particle Size Between CHD With High HDL Cholesterolemia and Normal Subjects With High HDL Cholesterolemia.

Discussion

A close relationship between the CETP activity and arteriosclerosis has been suggested. Quig et al. [15] reported that arteriosclerosis did not easily develop by high cholesterol in the rats with a low CETP activity and that on the contrary it easily developed in the rabbits with a high CETP activity. However, to our knowledge, no other group has evaluated the CETP activity in the patients with CHD.

As a CETP activity, radioactivity in LDL is usually measured after the incubation of the labeled HDL with LDL (Albers et al. [16]). Only the cholesteryl ester transfer from HDL to LDL can be measured by this method. But the cholesteryl ester transfer between the HDL and LDL occurred in both directions. Thus, the net cholesteryl ester transfer is important. We determined the net cholesteryl ester transfer in this study.

The cholesteryl ester transfer activity from HDL to LDL was observed to some extent in the normal subjects. In the CHD patients with high HDL cholesterolemia (more than 60 mg/dl), no cholesteryl ester transfer activity was confirmed. In the CHD patients with low HDL cholesterolemia (less than 60 mg/dl), on the contrary, a significant transfer activity was found (Fig. 1). These results show that the increase of cholesteryl ester transfer activity may have the risk of causing CHD in the patients with low HDL cholesterolemia. The low HDL cholesterolemia may be due to the increase of cholesteryl ester transfer activity. Nakanishi et al. [17] reported that the LDL cholesterol values correlated to the cholesterol ester transfer activity, providing a possibility that the increased atherogenic lipoproteins such as VLDL and LDL in response to the increase of transfer activity may participate in the onset of CHD.

HDL cholesterol values positively correlated to the rate of cholesteryl ester transfer activity, that is, higher HDL cholesterol values resulted in an increase of cholesteryl ester transfer activity from HDL to LDL in the normal subjects. This correlation was, however, negative in the CHD patients, which suggested a possibility of decreased HDL cholesterol values in response to the increased cholesteryl ester transfer activity.

Disturbed cholesteryl ester transfer activity from HDL to LDL was evident and the size of the HDL particle in both analyses using triglyceride and cholesterol monitoring was relatively large in the CHD patients with high HDL cholesterolemia as compared to the normal subjects with high HDL cholesterolemia. These results suggest that the large particle size of HDL may have the risk of causing CHD and that high HDL cholesterolemia may be due to the disturbed cholesterol transfer activity. Further studies may be necessary to clarify the relation between arteriosclerosis and cholesteryl ester transfer activity in the CHD patients with high HDL cholesterolemia.

In conclusion, we determined the cholesteryl ester transfer activity from HDL to LDL in the patients with CHD. In the CHD patients with low HDL cholesterolemia (less than 60 mg/dl), a significant transfer activity was found. In the patients with CHD, HDL cholesterol values reversely correlated to the rate of cholesteryl ester transfer activity. These results suggest that, at least in the CHD patients with low HDL cholesterolemia, an increase of cholesteryl ester transfer activity may have the risk of causing CHD and the low HDL cholesterolemia may be due to the increase of cholesteryl ester transfer activity.

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