Bull. Tokyo Med. Dent. Univ., 29: 131–137, 1982

ROLE AND METABOLISM OF HIGH DENSITY LIPOPROTEIN SUBFRACTIONS

—Analysis of serum HDL_2-cholesterol and HDL_3-cholesterol in patients with various diseases by high performance liquid chromatography—

BY

Akira TANAKA*1

ABSTRACT

In order to study the role and metabolism of high density lipoprotein (HDL) subfractions, the serum HDL_2-cholesterol (HDL_2-C) and HDL_3-cholesterol (HDL_3-C) were measured by the new method using high performance liquid chromatography in the normal subjects and patients with various diseases.

It was highly characteristic that the serum HDL_3-C levels of the patients with liver cirrhosis (LC) were remarkably lower than those of the normal subjects. The result suggests that HDL_3 may be produced in the liver.

Both the serum HDL_2-C and HDL_3-C levels were significantly lower in the patients with coronary heart disease (CHD) or cerebral thrombosis (CT) than in the normal subjects ($P<0.001$).

In the normal subjects, the changes in the serum HDL-cholesterol (HDL-C) levels were mainly due to those in the serum HDL_2-C levels. On the other hand, in the patients with atherosclerotic diseases (CHD, or CT) the changes in the serum HDL-C levels were attributed to those of both the serum HDL_2-C and HDL_3-C levels. So it is suggested that in the atherosclerotic diseases, in which the HCL-C is usually lower, the HDL_3-C also may play an important role in the regulation of the total HDL-C and its anti-atherogenetic effect.

INTRODUCTION

The role and metabolism of the high density lipoprotein (HDL) subfractions have recently become a subject of increasing interest, mainly due to the finding that the serum HDL-cholesterol (HDL-C) levels are inversely correlated with the incidence of coronary heart disease (CHD) [1–3].

However, the studies on the role and metabolism of the HDL subfractions have not yet progressed, because the ultracentrifugal method, which is the common method, consumes a long experimental time and a large amount of serum.

Hara et al. succeeded in developing for the first time the method of determination of HDL_2-cholesterol (HDL_2-C) and HDL_3-cholesterol (HDL_3-C) by high performance liquid chromatography (HPLC) using the aqueous gel permeation columns [4–5]. With this method,

*1 田中 明: Third Department of Internal Medicine (Chief: Prof. H. MAEZAWA), School of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
Received for publication, August 27, 1982.

the HDL_2-C and HDL_3-C can be measured directly with a small amount of whole serum (20 μl) in less than 50 minutes.

In the present study, the serum HDL_2-C and HDL_3-C levels were measured in the normal subjects and patients with various diseases by the HPLC method to investigate the role and metabolism of the HDL subfractions.

Materials and Methods

Blood was obtained after an overnight fast from 21 normal subjects (8 males, aged 43±15 yrs, M±SD; 13 females, 43±14 yrs), 23 patients with CHD (19males, 64±10 yrs; 4 females, 59±2 yrs), 7 patients with cerebral thrombosis (CT) (4 males, 66±9 yrs; 3 women, 56±12 yrs), 67 diabetics (29 males, 58±12 yrs; 38 females, 54±14 yrs) and 15 patients with liver cirrhosis (LC) (10 males 57±9 yrs; 5 females, 57±12 yrs). Normal subjects, who had no abnormalities in the serum triglyceride (TG), serum total cholesterol (TC), obesity index, blood sugar, blood pressure, ECG findings and liver function, were obtained from the health testing center. Patients with CHD had abnormal ECG findings (abnormal Q or ST depression) and had episodes of heart attacks. The 67 diabetics were 17 insulin-treated, 18 oral agent-treated, and 32 treated by diet only. Liver cirrhosis was pathologically diagnosed by liver biopsy.

Serum HDL_2-C and HDL_3-C levels were determined by the HPLC method (HLC 805, Toyo Soda Mfg. Co., Tokyo) according to the method of Hara et al. The quantitation of cholesterol was performed by the enzymatic reaction (Determiner TC 555, Kyowa Medics Co., Tokyo). The serum TG and TC levels were measured by the automated enzymatic method.

Results

In the normal subjects, the serum HDL_2-C and HDL_3-C levels in the males were compared with those in the females (Fig. 1). The age and serum TC level were matched between the males and females. The serum HDL_2-C levels in the females (closed circles) were significantly higher than those in the males (open circles) (35.2±14.8 v.s. 15.2±6.5 mg/dl M±SD. $P<0.002$). In contrast to the result, no difference was found between the serum HDL_3-C levels in the females and those in the males (24.6±7.8 v.s. 29.3±6.0 mg/dl. n.s.).

In the males, the serum HDL_2-C and HDL_3-C levels in the normal subjects and patients with CHD, CT and LC are shown in Fig. 2. The serum TC levels were matched among those groups except the LC group. Both the serum HDL_2-C and HDL_3-C levels were significantly lower in the patients with CHD than in the normal subjects (HDL_2-C: 9.3±3.1 v.s. 15.2±6.5 mg/dl, $P<0.01$; HDL_3-C: 19.1±3.9 v.s. 29.3±6.0 mg/dl, $P<0.001$). In the patients with CT, the same results were found as in the patients with CHD. However, it was highly characteristic that the serum HDL_3-C levels in the patients with LC were remarkably lower than those in the normal subjects, as shown in Fig. 2 (1.3±2.8 v.s. 29.3±6.0 mg/dl. $P<0.001$).

The relationship between the total HDL-C and HDL_2-C or HDL_3-C in all subjects except the patients with LC is shown in Fig. 3. By the serum levels of HDL-C, 119 subjects (21 normal, 67 diabetes, 22 CHD, 7 CT and 2 hypertriglyceridemia) were classified into 11 groups. Fig. 3 presents the mean levels of HDL_2-C on the left and those of HDL_3-C on the right, which are expressed as slanted bars, in the order of HDL-C levels rang-

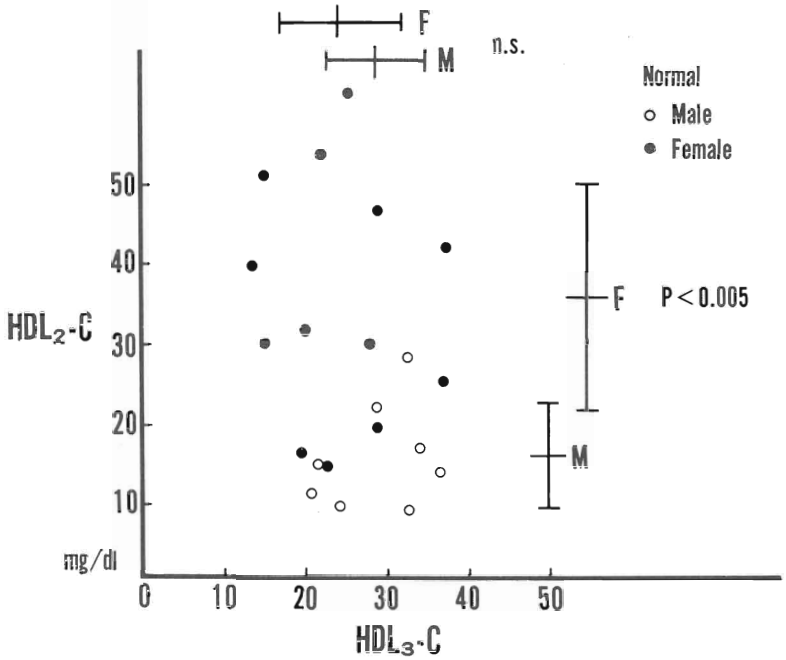

Fig. 1. Comparison of HDL_2-C and HDL_3-C between normal males and females.

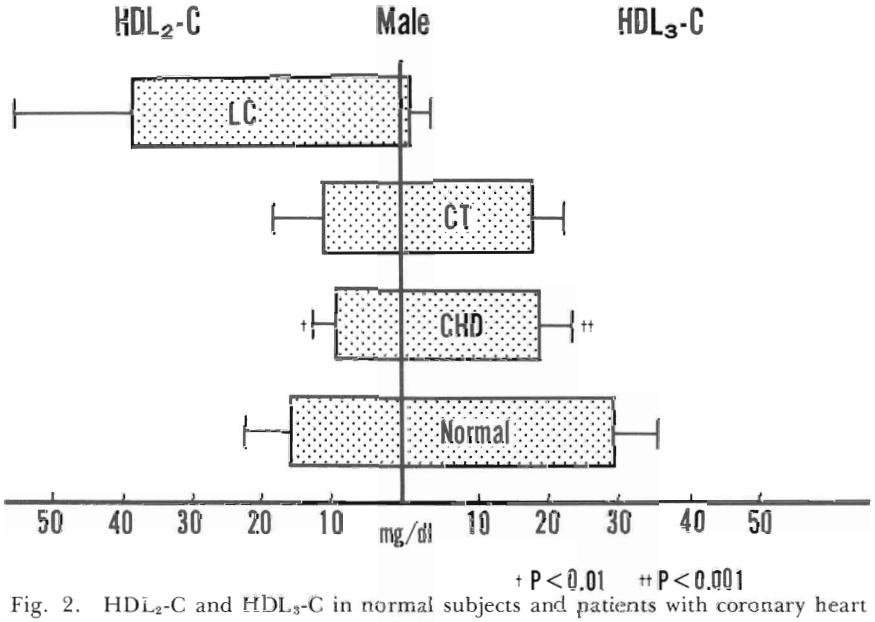

Fig. 2. HDL_2-C and HDL_3-C in normal subjects and patients with coronary heart disease (CHD), cerebral thrombosis (CT) and liver cirrhosis (LC).

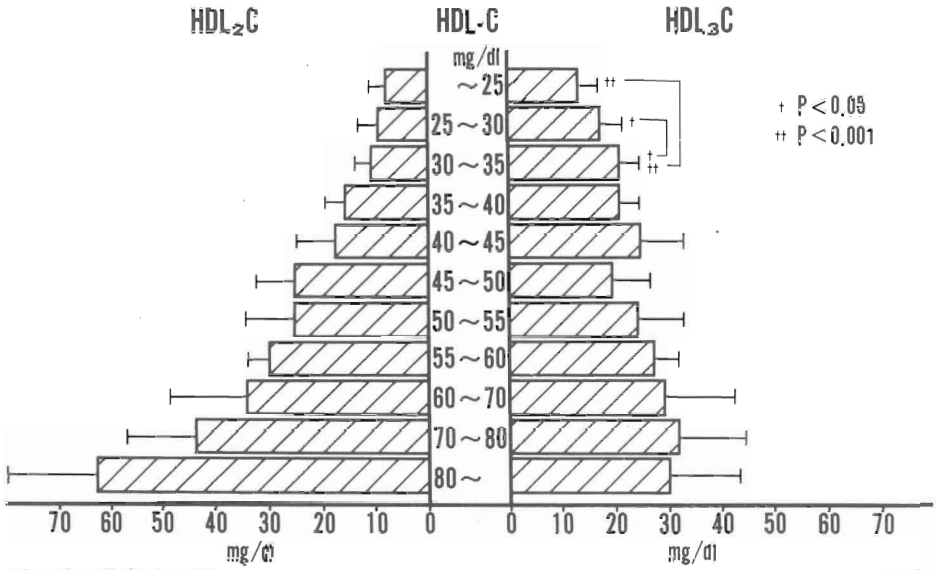

Fig. 3. Relationship between HDL_2-C and HDL-C (left), HDL_3-C and HDL-C (right) in all subjects except patients with LC (normal: 21, DM: 67, CT: 7, and hyperlipidemia: 2).

ing from 25 mg/dl or less to 80 mg/dl or more.

The HDL_2-C had a tendency to decrease in parallel with the HDL-C. On the other hand, no apparent change was observed in the HDL_3-C except the levels lower than 30 mg/dl of HDL-C. However, in the levels lower than 30 mg/dl of HDL-C, the HDL_3-C was also found to decrease in accordance with the decreasing levels of HDL-C. The HDL_3-C in the group ranged from 25 to 30 mg/dl, and in the group with less than 25 mg/dl of HDL-C revealed significantly lower serum levels than in the group ranging from 30 to 35 mg/dl of HDL/C.

Fig. 4. shows the relationship between the HDL-C and HDL_2-C or HDL_3-C in the normal subjects and patients with CHD. The HDL_2-C demonstrated a strong positive correlation with the total HDL-C in both the normal subjects and patients with CHD ($r=0.8494$, $P<0.01$, $r=0.9115$, $P<0.01$). The HDL_3-C showed a positive correlation with the total HDL-C in the patients with CHD ($r=$

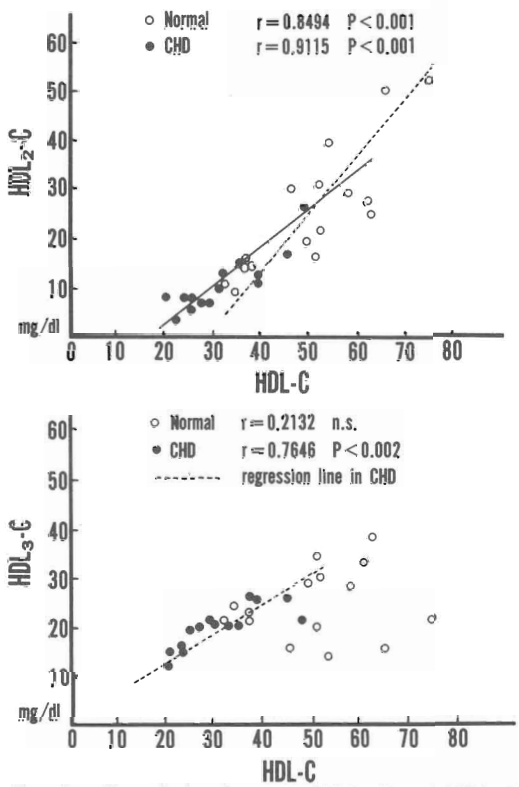

Fig. 4. Correlation between HDL_2-C and HDL-C (upper), HDL_3-C and HDL-C (lower) in normal and coronary heart disease (CHD).

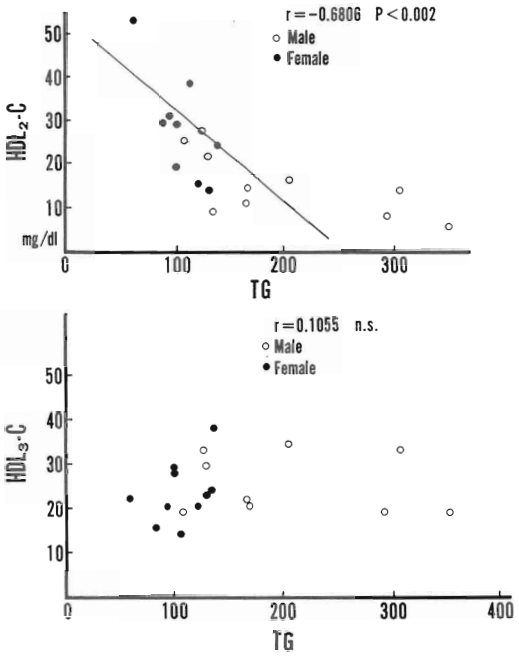

Fig. 5. Correlation between HDL_2-C and TG (upper), HDL_3-C and TG (lower) in normal subjects (including hypertriglyceridemia).

0.7646, $P<0.002$), however, in the normal sujects, there was no correlation between the HDL_3-C and total HDL-C ($r=0.2132$, n.s.).

The relationship between the TG and HDL_2-C or HDL_3-C is illustrated in Fig. 5. The HDL_2-C showed a negative correlation with TG ($r=-0.6806$, $P<0.002$). On the contrary, the HDL_3-C had no correlation with TG ($r=0.1055$, n.s.).

Discussion

In the present study, the serum HDL_2-C and HDL_3-C levels were measured by the method of Hara et al. using HPLC to investigate the role and metabolism of the HDL subfractions [4–5].

In the normal subjects, the HDL_2-C showed a strong positive correlation with the total HDL-C, whereas the HDL_3-C had no significant correlation with the total HDL-C (Fig. 4). And the elevation of the serum HDL_2-C levels in the normal females resulted in the differences in the serum total HDL-C levels between the males and females (Fig. 1). Furthermore, Wood et al. have reported that in the normal population study the increase in the HDL appears to be attributed almost to that in the HDL_2 component [6]. These results indicate that in the normal subjects the changes in the serum HDL levels are due mainly to those in the serum HDL_2 levels.

Anderson etal. measured the HDL subfractions, HDL_{2a}, HDL_{2b}, and HDL_3 in the normal subjects by the ultracentrifugal method and obtained the following results: (1) The serum HDL_3 levels show a small variation, whereas the serum HDL_2 levels exhibit a wide range of values. (2) Hence, the HDL_2 component is the major contributor to the inverse correlation of HDL-C with the prevalence of CHD. (3) And the contribution of the the HDL_3 component to any protective effect with respect to atherosclerosis is minimal [7].

However, these results are obtained from the study on the normal subjects. The results on the patients with atherosclerotic diseases (CHD, CT) are different from those of the normal subjects.

In this study, as shown in Fig. 3., in the range of less than 30 mg/dl of HDL-C, HDL_3-C also showed a decrease in accordance with the decreasing levels of HDL-C. Furthermore, as shown in Fig. 4., in the patients with atherosclerotic diseases, in which the serum HDL-C levels were usually lower, the HDL-C was correlated not only with the HDL_2-C but also with the HDL_3-C. These results indicate that in the atherosclerotic diseases, the changes in the serum HDL-C levels may be due to those of both the HDL_3-C and HDL_3-C. Therefore, it is concluded that the HDL_3-C may also play an important role in the regulation of HDL-C and

its anti-atherogenetic effect.

There was an inverse relationship between the serum TG levels and the serum HDL_2-C levels. Whereas no relationship was demonstrated between the serum TG levels and the serum HDL_3-C levels, as shown in Fig. 5. The results favour the hypothesis that the HDL_2 arises following the result of the catabolism of the TG-rich lipoproteins [8–9]. And it was highly characteristic that the patients with LC had remarkably lower HDL_3-C levels than the normal subjects. This evidence suggests that the HDL_3 may be produced in the liver [10]. Furthermore, it has been reported that the HDL_2 consists of two HDL_3 subunits plus additional lipids, and the HDL_3 may be converted into HDL_2 [11]. These results suggest that the conversion of HDL_3 to HDL_2, the lipolytic process of the TG rich-lipoprotein and the HDL_3 production in the liver may play an important role in the regulation of the total HDL levels [12]. Considering the closer metabolic relationship between the HDL_2-C and HDL_3-C, it is not likely that the HDL_2 may be only a subfraction preventing the development of atherosclerosis. The HDL_3 fraction seems to have a reserve function for compensating the decrease of the HDL_2 fraction. Further investigation is necessary to clear the role and metabolism of the HDL subfractions.

Acknowledgement

The author expresses his deep thanks to Prof. H. Maezawa of this department and to Dr. M. Okazaki and Prof. I. Hara of the Laboratory of Chemistry, Department of General Education, Tokyo Medical and Dental University, for their useful advices and suggestions.

References

1) Castelli, W. P., Doyle, J. T., Gordon, T., Hames, C. G., Hjortland, M. C., Hulley, S. B., Kagan, A., and Zukel, W. J.: HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulations, 55: 767 (1977).
2) Miller, N. E., Forde, O. H., Thelle, D. S., and Mjos, O. D.: The Tromso heart study. High-density lipoprotein and coronary heart disease. A prospective case-control study. Lancet, 1: 965 (1977).
3) Gordon, T., Castelli, W. P Hjortland, M. C., Kannel, W. B., and Dawber, T. R.: High density lipoprotein. As a protective factor against coronary heart disease. Am. J. Med., 62: 707 (1977).
4) Hara, I., Okazaki, M., and Ohno, Y.: Rapid analysis of cholesterol of HDL and LDL in human serum by high performance liquid chromatography. J. Biochem., 87: 1863 (1980).
5) Okazaki, M., and Hara, I.: Analysis of cholesterol in high density lipoprotein subfractions by high performance liquid chromatography. J. Biochem., 88: 1215 (1980).
6) Wood, P. D., and Haskell, W. L.: The effect of exercise on plasma high density lipoproteins. Lipids, 14: 417 (1979).
7) Anderson, D. W., Nichols, A. V., Pan, S. S., and Lindgren, F. T.: High density lipoprotein distribution. Atherosclerosis, 29: 161 (1978).
8) Eisenberg, S.: Effect of temperature and plasma on the exchange of apolipoproteins and phospholipids between rat plasma very low and high density lipoprotein. J. Lipid Res., 19: 229 (1978).
9) Patch, J. R., Gotto, Jr., A. M., Olivecrona, T., and Eisenberg, S.: Formation of high density lipoprotein$_2$-like particles during lipolysis of very low density lipoprotein in vitro. Proc. Nat. Acad. Sci. USA, 75: 4519 (1978).
10) Okazaki, M., Hara, I., Tanaka, A., Kodama, T., and Yokoyama, S.: Decreased serum HDL_3 cholesterol levels in cirrhosis of the liver. N. Eng. J. Med., 304 (26): 1608, (1981).
11) Grow, T. E., and Fried, M.: Interchange of apoprotein components between the human plasma high density lipoprotein subclasses HDL_2 and HDL_3 in vitro. J. Biol. Chem. 243: 8034 (1978).
12) Tanaka, A., Uchimura, I., Sugiyama, H., Oda, H., and Maezawa, H.: Analysis of serum high density lipoprotein-cholesterol reg-

ulation by HDL_2 and HDL_3-C in health and chronic liver disease, using high performance liquid chromatography (in Japanese). J. Japan. Soc. Intern. Med. 70: 1691 (1981).