Hiroshima Journal of Medical Sciences
Vol. 32, No. 1, March, 1983
HIJM 32-12

Change in Constituents of High Density Lipoprotein and its Subfractions in Circulation from Hepatic Vein to Femoral Artery*)

Koki TAKATA, Masaharu YAMAMOTO, Fumiaki HINO,
Hideo MATSUURA, Hiroyuki KUROGANE and Goro KAJIYAMA.

The First Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan

(Received December 4, 1982)

Key words: *HDL, HDL_2, HDL_3, Hepatic vein, Femoral artery*

ABSTRACT

The difference in HDL-lipid constituents was examined in sera from the hepatic vein and femoral artery. In HDL, HDL_2 and HDL_3 the most pronounced difference existed in the triglyceride concentration which was significantly higher in the femoral artery as compared to the hepatic vein. The cholesterol and phospholipid concentrations of HDL_2 alone but not of HDL_3 were also significantly higher in the femoral artery than hepatic vein. The concentrations of apo A-I and A-II, on the other hand, remained unaltered in the blood circulation. These results, in combination with the finding of a significant decrease in triglycerides of VLDL and LDL fraction (d.=1.006−1.063) in the femoral artery as compared to the hepatic vein suggest that the accumulation of triglycerides in HDL is due to their transfer from other triglyceride-rich lipoprotein and the accumulation of HDL_{2b} occurs in the blood stream from the hepatic vein to the femoral artery.

INTRODUCTION

A number of recent epidermiological data and in vitro experiments suggest that HDL (High Density Lipoprotein) has antiatherogenesity either transporting cell membrance cholesterol from peripheral (atheromatous) tissues to the liver in humans and animals or suppressing the net increment in cell sterol contents indured by low density lipoprotein (LDL)[3,6,7,11]. HDL is divided according to the difference in density by ultracentrifugation into two subclasses, HDL_2 (d.=1.063−1.125) and HDL_3 (d.=1.125−1.210). HDL_2 is considered to be converted from HDL_3 with the aid of triglyceride-rich lipoprotein and lipoprotein lipase[14]. This conversion from HDL_3 to HDL_2 is accompanied by transportation of peripheral cholesterol to the liver, that is, HDL_3 is transformed into HDL_2 by incorporating tissue cholesterol and increasing its diameter. The difference in lipid and lipoprotein compositions between HDL_2 and HDL_3 also supports the above hypotheses.

Therefore, HDL seems to undergo a large constitutional change in the blood stream. In this study we analyzed the main lipid and apoprotein constituents of serum HDL from the hepatic vein and femoral artery to estimate their difference between sera from these vessels which are directly connected to lipoprotein-metabolizing organ, i. e., the liver.

PATIENTS

A. Effect of heparin on HDL and its subfraction lipids (preliminary experiment)

Three normal volunteers were injected intravenously with 13 u/kg of heparin to see if a small amount of heparin filled in a catheter as an anticoagulant causes changes in their

*) 高田耕基，山本正治，日野文明，松浦秀男，鍈　寛之，梶山梧朗：肝静脈一股動脈間の血流中に起る高比重リポ蛋白及びその亜分画構成成分の変化

serum levels. HDL fractions were analyzed before and twenty minutes after injection.

B. Analysis of serum lipoprotein in hepatic vein and femoral artery

Catheterization was undertaken using a 7F Cournand cardiovascular catheter filled with 1000 μ/dl heparin solution (in saline) to prevent blood coagulation in a tube. The amount of heparin solution used before blood sampling was minimized to 2-10 ml (20-100 u heparin) to avoid the influence of heparin on serum lipid and lipoprotein levels, after preliminary experiment for confirming the effect of 13 u/kg heparin injection on serum lipoprotein concentrations in these 3 volunteers. Immediately or several minutes after heparin injection, blood was drawn from the femoral artery using a 21 gauge needle by direct puncture. Twenty milliliters of blood from three healthy volunteers were collected before and twenty minutes after heparin and serum was immediately prepared and used for analysis of HDL_2 and HDL_3 lipid and lipoprotein concentrations.

Sera from the hepatic vein and femoral artery were obtained from eleven patients, five males and six females aged 20 to 72 who were admitted for cardiac catheterization. Out of eleven patients without left to right shunt, eight were valvular diseases, one hypertrophic cardiomyopathy, one WPW syndrome and one sick sinus syndrome. All had no hyperlipidemia when examined by routine tests immediately after admission.

LIPOPROTEIN ANALYSES

Sera from two vessels were obtained at catheter examination. Combined VLDL (Very Low Density Lipoprotein) and LDL, and HDL were prepared according to the method reported by Havel et al.[8].

Analysis of HDL_2 and HDL_3 was performed as described previously[9]. Total cholesterol was determined by the enzymatic method[1] using an autoanalyzer. Triglycerides were determined using lipoprotein lipase for hydrolysis[18] (TG Kit, Ono Pharmaceutical Co., Japan). Phospholipids were also determined by the enzymatic method described by Takayama et al.[19] (Nippon Shoji Japan). Apoprotein A-I (apo A-I) and A-II (apo-II) were determined by electroimmunoassay as reported by Curry et al.[4] (Daiichi Chemical Co., Japan).

STATISTICAL ANALYSIS

The values of lipid and apoprotein concentrations were analyzed by Wilcoxon test.

RESULTS

I. Preliminary experiment (effect of intravenous heparin on HDL and its subfraction lipid) (Table 1)

Table 1. HDL and its subfraction lipid concentration before and 20 minutes after intravenous heparin

	M Y male		M N male		K T male	
Heparin	before	after	before	after	before	after
HDL-C	52	51	48	51	41	42
-TG	14	9	10	14	22	10
-PL	109	105	100	100	91	92
HDL_2-C	10.9	14.6	7.9	8.6	8.6	4.8
-TG	5.6	3.8	2.6	1.5	3.9	1.9
-PL	13.2	19.5	11.2	11.3	14.0	7.2
HDL_3-C	41.0	36.4	40.1	42.3	32.3	37.2
-TG	8.4	4.8	6.9	12.4	18.2	7.7
-PL	66.3	63.2	63.8	65.6	58.2	62.4

(mg/dl)

As listed in Table 1 no noticeable change was noted in HDL, HDL_2 and HDL_3 lipid constituents, except for HDL_2-triglycerides which showed a lower concentration in the serum after heparin injection as compared to the serum before heparin injection.

II. Lipids and apoproteins in HDL and its subfractions of serum from hepatic vein and femoral artery (Table 2)

1) Lipids and apoproteins in HDL (d.=1.063 -1.210)

There were no significant differences in lipid and apoprotein concentrations of HDL fractions in serum between the hepatic vein and femoral artery, except for triglycerides ($p<0.01$) which revealed significantly higher concentrations in serum from the femoral artey as compared to the hepatic vein.

2) Lipids and apoproteins in HDL_2 (d.=1.063 -1.125)

Cholesterol ($p<0.05$), triglyceride ($p<0.01$) and phospholipid ($p<0.05$) concentrations were significantly higher in the femoral artery as

Table 2. Main constituents of HDL and its subfractions

		HDL	HDL_2	HDL_3
Hepatic Vein	Cholesterol	43.8± 8.5	41.6± 6.5	32.3± 6.7
	Triglycerides	14.7± 7.5	4.2± 2.6	10.5± 5.7
	Phospholipids	86.5±14.3	15.3± 7.5	48.3±12.1
	A-I	87.1±24.9	15.6± 9.8	71.7±26.3
	A-II	22.5± 8.1	2.7± 3.0	18.8± 8.1
Femoral Artery	Cholesterol	44.9± 8.4	15.9± 7.2*	29.0± 6.3
	Triglycerides	21.1± 7.8**	8.0± 3.3**	13.1± 6.8*
	Phospholipids	88.4±13.7	21.6± 8.9*	45.2±12.4
	A-I	82.8±24.0	19.3± 9.6	62.5±25.5
	A-II	21.5± 5.2	3.8± 2.1	16.4± 5.6

(mg/dl)

*$p<0.05$ **$p<0.01$ statistically significant between hepatic vein and femoral artery

compared to the hepatic vein.

But there was no significant difference in apoprotein A-I and A-II concentrations in HDL_2 between the hepatic vein and femoral artery.

3) Lipids and apoproteins in HDL_3 (d.=1.125 −1.210)

There were again no statistically significant differences in lipid and apoprotein concentrations between the hepatic vein and femoral artery, except for triglycerides which was higher in the femoral artery as compared to the hepatic vein ($p<0.05$).

Ⅲ. Total, VLDL and LDL-lipids (d.=1.006−1.063)

As listed in Table 3, there were no significant differences in total serum lipid concentrations between the hepatic vein and femoral artery. However, a statically significant decrease was observed in VLDL and LDL triglyceride concentration (d.=1.006−1.063) from the femoral artery as compared to that from the hepatic vein.

DISCUSSION

The most pronounced difference was found in the triglyceride concentrations both in HDL_2 and HDL_3 between the sera from the hepatic vein and femoral artery. It is well known that heparin causes a dramatic change in a serum triglyceride concentration particularly in triglyceride-rich lipoproteins, by release of lipoprotein lipase[10,15]. However, the results of the preliminary experiment clearly disclosed that the difference in triglycerides was not due to heparin flush for prevention of blood coagulation in the present experiment, because the triglyceride concentration was higher in the femoral artery than in the hepatic vein (entirely opposite results to the change in the triglyceride level in the preliminary experiment), and heparin was flushed from a catheter and blood was drawn from the hepatic vein shortly before drawing blood from the femoral artery in most of the patients. If it is assumed that the majority of HDL in the hepatic vein was secreted from the liver and that the artery (femoral artery) mainly contains HDL almost ready to be transferred and incorporated into the liver after passing through various metabolic effects in the blood stream, the difference in the triglyceride concentration between the two vessels must occur during circulation in the peripheral vessels.

Investigations of plasma triglycerides have been mostly concentrated on their transport in chylomicrons and VLDL, while the metabolism of triglycerides in HDL has been given little attention. However, recent investigations clarified that (1) triglycerides in HDL may have their origin in VLDL, since the transfer of triglycerides from VLDL to HDL has been observed in vitro[13,16,17], the fatty acid composition of HDL triglycerides is similar to that of other lipoproteins[12] and furthermore, ingestion of large meals of safflower or olive oil produces a change in the fatty acid composition of triglycerides in all lipoprotein fractions, including HDL[12], (2) there is a rapidly turning-over pool of HDL triglycerides resulting from the transfer from VLDL and a slowly turning-

Table 3. Serum and VLDL and LDL (d. 1.006-1.063) lipids in hepatic vein and femoral artery

		Serum	VLDL and LDL (d. 1.006-1.063)
Hepatic vein	Cholesterol	184.4±35.2	140.9±34.4
	Triglycerides	99.1±28.1	84.4±23.8
	Phospholipids	185.9±24.7	99.5±16.7
Femoral artery	Cholesterol	186.1±37.5	141.2±35.3
	Triglycerides	93.7±23.0	72.3±20.4**
	Phospholipids	187.5±28.2	98.2±19.2

(mg/dl)

**$p<0.01$ Statistically significant between hepatic vein and femoral artery

over pool which may or may not have originated from VLDL[5] and (3) HDL triglycerides are correlated positively and significantly with the concentration of VLDL triglycerides[2]. On the basis of these results from recent investigations, we can estimate that the difference in HDL_2 and HDL_3 triglyceride concentrations between the two vessels is due to the transfer of triglycerides from VLDL to a HDL-rapidly turning-over pool in the blood circulation from the hepatic vein to femoral artery.

Therefore, this transfer seemed to partially contribute to the decrease of triglycerides in LDL and VLDL (as shown in Table 3), although the majority of triglycerides in these fractions is catabolized by lipoprotein and hepatic lipases in the blood stream. Although it has been reported that radioactive decay was more slowed in HDL than in VLDL[5], what is the subsequent metabolism of HDL-triglycerides whether it is cleaned from the plasma or again transferred to another, the accumulation of triglycerides in HDL more in the femoral artery than in the hepatic vein seems to suggest that the liver is one of the catabolic sites of triglycerides transferred from HDL.

In contrast to triglycerides the increase of cholesterol and phospholipids occurred in HDL_2 alone but not in HDL_3 during blood circulation from the hepatic vein to femoral artery. According to the recent study by Andersson et al.[2], HDL is not only divided simply into HDL_2 and HDL_3 but also HDL_2 is further subdivided into HDL_{2a} (d.=1.100−1.125) and HDL_{2b} (d.=1.063−1.100) by analytical centrifugation. HDL_{2b} has a larger particle diameter (10.8−12 nm) containing more cholesterol, tryglycerides and phospholipids and less protein than HDL_{2a} (diameter range of 9.7−10.7 nm)[2]. In our present analysis, there was a remarkable increase of cholesterol, triglyceride and phospholipid concentrations in HDL_2 but there was no significant increase of apoprotein A-I and A-II. This unbalanced and preferential incerase of HDL_2-lipid constituents may suggest that the relative increase of HDL_{2b} to HDL_{2a} occurs during circulation of lipoproteins in blood from the hepatic vein to the femoral artery. Therefore, we failed to indicate that the apparent increase of serum HDL_2 occurred in the femoral artery during blood circulation, though there was a very small but no significant increase of apoproteins A-I and A-II whose significant and parallel increase with lipid constituents may indicate the absolute increase of HDL_2 concentration in the serum of femoral artery.

REFERENCES

1. **Allain, C. C., Poon, L. S., Chan, S. G., Richmond, W. and Fu, P. C.** 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. **20** : 470-475.
2. **Anderson, D. W., Nichols, A. V., Rorte, T. M. and Lindgren, F. T.** 1977. Particle distribution of human serum high density lipoproteins. Biochim. Biophys. Acta **493** : 55-68.
3. **Carew, T. E., Koschinsky, T., Hayes, S. B. and Steinberg, D.** 1976. A mechanism by which high-density lipoproteins may slow the atherogenic process. Lancet **i** : 1315-1317.
4. **Curry, M. D., Alaupovic, P. and Sueuram, C. A.** 1976. Determination of apolipoprotein A and its constitutive A-I and A-II polypeptides by separate electroimmunoassay. Clin. Chem. **22** : 315-322.
5. **Farquhar, J. W., Gross, R. C. and Wagner, R. M.** 1965. Validation of an incompletely coupled twocompartment non-recycling catenary model for turnover of liver and plasma triglyceride in man.

J. Lipid Res. 6 : 119-134.

6. **Clomset, J. A.** 1970. Pysiological role of lecithin-cholesterol acyltransferse. Amer. J. Clin. Nutr. 23 : 1129-1136.
7. **Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. and Dawber, T. R.** 1977. High density lipoprotein as a protective factor aginst coronay hear diseases. The Framinghan Study Amer. J. Med. 63 : 707-714.
8. **Havel, R. J., Eder, H. A. and Bragdon, J. H.** 1955. The distribution and chemical composition of ultacentrifugally separated lipoproteins in human serum. J. Clin. Invest. 34 : 1345-1353.
9. **Kajiyama, G., Nakagawa, M., Takata, K., Nakao, S. and Miyoshi, A.** 1981. Determination of cholesterol and phospholipids of HDL_2, HDL_3 and VHDL in patients with atherosclerosis and diabetes mellitus, Hiroshima J. Med. Sci. 30 : 175-182.
10. **Levy, R. I., Lees, R. S. and Fredrickson, D. S.** 1966. The nature of pre-beta (very low-density) lipoproteinis. J. Clin. Invest. 45 : 63-77.
11. **Miller, G. J. and Miller, N. E.** 1965. Plasma high density lipoprotein concentration and development of ischemic heart diseases. Lancet i : 16-19.
12. **Nichols, A. V., Rehnborg, C. S., Lindgren, F. T. and Wills, R. D.** 1962. Effects of oil ingestion on lipoprotein fatty acids in man. J. Lipid Res. 3 : 320-326.
13. **Nichols, A. V. and Smith, L.** 1965. Effects of very low density lipoproteins on lipid transfer in incubated serum. J. Lipjd Res. 6 : 206-210.
14. **Patch, J. R., Gotto, A. M., Olivercrona, T. and Eisenberg, S.** 1978. Formation of high density lipoprotein2-like particles during lypolysis of very low density lipoproteins in vitro. Proc. Natl. Acad. Sci. 75 : 4519-4523.
15. **Porte, D. Jr. and Bierman, E. L.** 1969. The effect of heparin infusion on plasma triglyceride in vivo and in vitro with a method for calculating triglyceride turnover. J. Lab. Clin. Med 73 : 631-648.
16. **Quarfordt, S. H., Boston, F. and Hildermen, H.** 1971. Transfer of triglyceride between isolated human lipoproteins. Biochim. Acta 231 : 290-294.
17. **Rehnborg, C. S. and Nichols, A. V.** 1964. The fate of cholesteryl esters in human serum incubated in vitro at 38°C. Biochim. Biophys. Acta 84 : 596-603.
18. **Takahashi, J.** 1974. The determination of serum triglyceride by the enzymatic hydrolysis. Jap. J. Clim. Chem. 2 : 425-432 (English abstract).
19. **Takayama, M., Itoh, S., Nagasaki, T. and Tanimizu, I.** 1977. A new enzymatic method for determination of serum choline-containing phospholipis. Clin. Chim. Acta 79 : 93-98.