Hiroshima Journal of Medical Sciences
Vol. 30, No. 4, December, 1981
HIJM 30-37

HDL AND ITS SUBFRACTIONS IN PATIENTS WITH CHRONIC LIVER DISEASES*)

By

Goro KAJIYAMA, Koki TAKATA, Itaru HORIUCHI,
Ken OYAMADA and Akima MIYOSHI

1st Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan

(Received August 17, 1981)

ABSTRACT

Serum lipids, lipids (cholesterol, triglycerides and phospholipids) and apo-A in d.>1.063 fraction, HDL_2, HDL_3 and VHDL of 10 normal subjects and 34 patients with chronic liver cliseases (chronic aggressive hepatitis 2A and 2B, and liver cirrhosis in the compensated and decompensated stages) were analyzed.

The analysis of d.>1.063 and its subfractions (HDL_2, HDL_3 and VHDL) was achieved by relatively simple procedures with combination of precipitation method and ultracentrifugal technique.

Many lipids and apo-A in HDL decreased in these diseases, particularly in liver cirrhosis in the decompensated stage. Triglycerides were exceptional and tended to increase. Lipids and apo-A in both HDL_2 and HDL_3 tended to decrease but the magnitude of their decrease in HDL_3 exceeded that in HDL_2. Phospholipids in VHDL also decreased in these patients.

The above results demonstrated, therefore, that the decrease in HDL-cholesterol and apo-A is mainly due to their decrease in HDL_3, and the decrease in phospholipids in d.>1.063 is mainly due to their decrease in both HDL and VHDL fractions. These results totally differed from what was seen in atherosclerotic patients in whom the decrease in d.>1.063 fraction chiefly resulted from the decrease in HDL_2 fraction.

Among the patients with liver diseases there was no significant inverse correlation between serum triglycerides and HDL-cholesterol, as observed in atherosclerotic patients.

The LCAT activity and VHDL-phospholipids that contain plentiful lysolecithin produced during the LCAT reaction, were also not correlated.

INTRODUCTION

HDL (high density lipoprotein) is known to be synthesized both in the liver[1] and intestine[2] and excreted into the blood stream. HDL is further known to be synthesized by lipoprotein and hepatic lipase during the degradation of triglyceride-rich lipoprotein[3].

Therefore, the liver can be estimated as very important as the intestine for the regulation of

*) 梶山梧朗，高田耕基，堀内　至，小山田健，三好秋馬：慢性肝疾患の HDL およびその亜分画

serum HDL level.

HDL is found in the discoidal nascent forms in the incubation medium after liver reflux of rats[4] and in two different spheric forms (HDL_2 and HDL_3) in the blood stream.

HDL, particularly cholesterol in HDL (HDL-cholesterol), decreases in patients with ischemic heart diseases[5] and phospholipids in HDL decreases in patients with ischemic cerebrovascular diseases[6].

The decrease in HDL_2 is significant when compared with the decrease in HDL_3 in these atherosclerotic patients[7,8].

The decrease in HDL_2 is suggested to be mainly due to the degradation of triglyceride-rich lipoproteins being inhibited in sera of these patients, because triglyceride-rich lipoproteins and HDL-cholesterol are inversely correlated[9] and, in addition, the HDL_2-like particles are produced in the incubation mixture of HDL_3 and triglyceride-rich lipoproteins[10].

In the present experiment, lipid and lipoprotein constituents of HDL and VHDL (very high density lipoprotein) were analyzed in patients with chronic liver diseases to investigate the influence of these parenchymal liver damages on the lipid and apoprotein constituents, especially on HDL subfractions.

METHODS

Lipids and HDL constituents of sera from 10 normal subjects (6 males and 4 females) and 34 patients with chronic liver diseases were analyzed.

The normal controls were not receiving drug therapy and had no symptoms, signs, or history of liver disease. They had normal conventional liver function tests. Out of 34 patients, 8 had chronic aggressive hepatitis 2A; 8 had 2B;[11] 12 had cirrhosis in the compensated and 6 had cirrhosis in the decompensated stage. Diagnosis of the patients were made on the basis of clinical symptoms, liver function tests, liver biospy and in some cases with laparoscopy.

The d.$>$1.063 fraction which contains the HDL_2, HDL_3 and VHDL subfractions was separated by precipitating d.$<$1.063 fraction according to the Burnstein's method[12]. Fractionation of HDL_2, HDL_3 and VHDL was performed as described previously[13].

To be more concrete, after low and very low density lipoproteins (d.$<$1.063) were precipitated by adding 4% sodium phosphotangstate and 2M $MgCl_2$ to serum, ultracentrifugation of the supernate was repeated twice with adjustment of density each time.

Cholesterol[14], triglycerides[15] and phospholipids[16] were determined in each fraction by the enzyme method.

Apo-A (A-I+A-II) was determined by rocket electrophoresis. Apoprotein-A standard and antiserum were purchased from Hoechst Japan Co.

The determination of LCAT activity of serum from 10 normal subjects and 26 patients with chronic liver diseases was achieved by the colorimetric methods described by Nagasaki and Akanuma[17] which was based on the determination of initial esterification rate of free serum cholesterol.

RESULTS

1. *Serum lipids and LCAT activity in normal subjects and patients with chronic liver diseases:*

Total serum cholesterol decreased in liver cirrhosis, regardless of the stage being compensated or decompensated ($p<0.05$). The triglyceride and phospholipid levels rose significantly in chronic aggressive hepatitis 2B ($p<0.01$, $p<0.001$).

The LCAT activity was inhibited in chronic aggressive hepatitis 2B and liver cirrhosis in the decompensated stage ($p<0.05$), although there was no statistically significant difference in the activity of chronic aggressive hepatitis 2B from that of the normal subjects. (Table 1)

2. *Lipid and apo-A concentrations of d.$>$1.063 fraction in normal subjects and patients with chronic liver disease:*

The d.$>$1.063 fraction includes HDL and VHDL fractions. The low cholesterol level was observed in chronic aggressive hepatitis 2B and liver cirrhosis both in the compensated and decompensated stages ($p<0.01$). On the contraty, the triglyceride level of this fraction was observed risen in chronic aggressive hepatitis 2A and liver cirrhosis in the compensated stages ($p<0.01$).

The phospholipid level was apparently low in liver cirrhosis in the decompensated stage. Apo-A was low in chronic aggressive hepatitis 2B and liver cirrhosis in both the compensated

Table 1. Serum lipids and LCAT activity in normal subjects and patients with chronic liver diseases

		Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	LCAT (nmole/ml/hr. 37°C)
Normal subjects	(10)	198±23	80.1±18.5	206±42	92.2±36.6
CAH (2A)	(8)	198±41	96.9±21.3	215±33	103.6±52.6
CAH (2B)	(8)	202±42	119.1±32.3**	223±32***	55.1±51.7
LC (Comp)	(12)	160±26**	107.4±65.8	222±83	71.4±55.3
LC (Decomp)	(6)	144±38**	106.2±62.3	183±55	42.5±51.4*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ statistically significant against normal subjects

Table 2. Lipid and Apo-A concentrations of d.>1.063 fraction in normal subjects and patients with chronic liver disease

(mg/dl)

		Cholesterol	Triglycerides	Phospholipids	Apo-A
Normal subjects	(10)	55.9±12.2	16.8± 4.5	102.8±16.6	185±22
CAH (2A)	(8)	52.1±12.9	24.3± 4.9**	101.5±17.1	174±33
CAH (2B)	(8)	41.9± 7.1**	22.0± 7.6	92.0±11.7	149±16**
LC (Comp)	(12)	39.9±10.2**	29.4±10.2**	88.4±18.9	110±37***
LC (Decomp)	(6)	29.8±11.1**	22.7± 9.2	62.8±17.7***	118±51*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ statistically significant against normal subjects

Table 3. Lipid and Apo-A concentrations in HDL_2 ($1.063<d.<1.125$) fraction in normal subjects and patients with chronic liver diseases

(mg/dl)

		Cholesterol	Phospholipids	Apo-A
Normal subjects	(10)	16.9± 8.5	29.6±15.3	53.0±16.7
CAH (2A)	(8)	16.9± 7.4	23.0± 8.9	47.6±18.7
CAH (2B)	(8)	11.8± 3.6	19.4± 6.4	42.8±10.3
LC (Comp)	(12)	17.7±11.3	30.6±18.0	35.4±21.2
LC (Decomp)	(6)	11.5± 4.9	19.8± 7.6	23.1±10.2**

** $p<0.01$ statistically significant against normal subjects

Table 4. Lipid and Apo-A concentrations in HDI_3 ($1.125<d.<1.210$) fraction in normal subjects and patients with chronic liver diseases

(mg/dl)

		Cholesterol	Phospholipids	Apo-A
Normal subjects	(10)	37.3±11.2	62.5±18.8	122.8±51.2
CAH (2A)	(8)	35.2± 8.9	53.3±10.5	125.0±19.6
CAH (2B)	(8)	30.9± 9.7	53.7±12.8	104.9±15.6
LC (Comp)	(12)	23.9± 7.8**	47.0±15.0*	74.7±28.8*
LC (Decomp)	(6)	19.4± 6.5**	37.5± 9.3**	74.1±28.5*

* $p<0.05$, ** $p<0.01$ statistically significant against normal subjects
CAH: Chronic active hepatitis, LC: Liver cirrhosis,
Comp.: Compensated, Decomp.: Decompensated.

and decompensated stages. (Table 2)

3. *Lipid and apo-A concentrations in HDL (1.063<d.<1.125) fraction in normal subjects and patients with chronic liver diseases:*

The cholesterol and phospholipid concentrations of this fraction showed a tendency to decrease in chronic aggressive hepatitis 2B and liver cirrhosis in the decompensated stage, with-

out, however, a statistical significance against the normal subjects. Apo-A also tended to decrease in these diseases, though there was statistical significance only in liver cirrhosis in the decompensated stage against the normal subjects ($p<0.01$). (Table 3)

4. *Lipid and apo-A concentrations in HDL ($1.125<d.<1.210$) fraction in normal subjects and patients with chronic liver diseases:*

When comparison of liver cirrhosis was made between the compensated and decompensated stages, the decrease in cholesterol and phospholipids was more moderate in the former stage, though not statistically significant, than in the latter. (Table 4)

The cholesterol, phospholipid and apo-A levels of this fraction were also low in these diseases except in chronic aggressive hepatitis 2A. However, it was only in liver cirrhosis in the compensated and decompensated stages that showed statistically significant decrease against the normal subjects. The lipids and apo-A levels of this fraction were also low in these disease except in chronic aggressive hepatitis 2A. Especially liver cirrhosis and statistically significant low cholesterol ($p<0.05$), phospholipid ($p<0.05$ and $p<0.01$) and apo-A($p<0.05$) concentrations in compensated and decompensated stage.

5. *Phospholipid concentration in VHDL ($d.>1.210$) fraction in normal subjects and patients with chronic liver diseases:*

Phospholipids in VHDL was significantly low in chronic aggressive hepatitis 2B and liver cirrhosis in the decompensated stage ($p<0.05$). (Table 5)

6. *Relationship between serum triglycerides and cholesterol in $d.>1.063$ fraction:*

As shown in Fig. 1, there was no statistical signifficance in the correlation between serum triglycerides and cholesterol in $d.>1.063$ fraction in 10 normal subjects and 34 patients with chronic liver diseases. (Fig. 1)

Table 5. Phospholipid concentration in VHDL ($d.>1.210$) fraction in normal subjects and patients with chronic liver diseases

(mg/dl)

		Phospholipids in VHDL
Normal subjects	(10)	20.8±6.3
CAH (2A)	(8)	22.5±5.8
CAH (2B)	(8)	14.9±5.3*
LC (Comp)	(12)	16.9±9.6
LC (Decomp)	(6)	12.0±6.0*

*P>0.05 Statistically significant against normal subjects

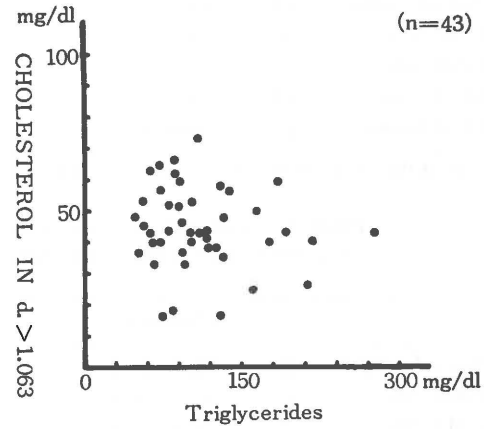

Fig. 1. Relationship between serum triglycerides and cholesterol in $d.>1.063$ fractions

7. *Relationship between phospholipid concentration in VHDL and LCAT activity:*

As shown in Fig. 2, no statistically significant correlation was observed between the phospholipid concentration in VHDL and the LCAT activity in 10 normal subjects and 26 patients with chronic liver diseases. (Fig. 2)

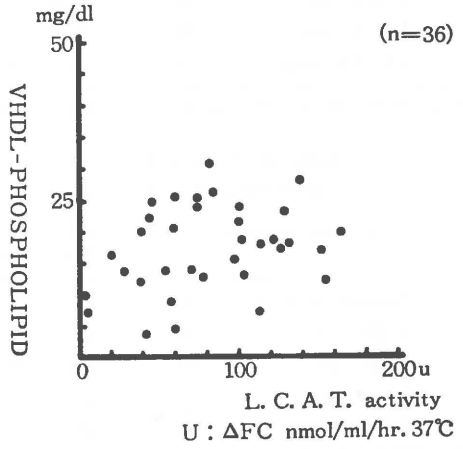

Fig. 2. Relationship between phospholipid concentration in VHDL and LCAT activity

DISCUSSION

Serum lipids are known to alter in various acute and chronic hepatobiliary diseases and

have been frequently adopted as a clinical indicator of hepatibiliary functions. Among serum lipids, total serum cholesterol is lipids of high diagnostic reliability for liver cirrhosis. The slight or moderate elevation of serum triglycerides is often observed in chronic hepatitis which is considered to be caused by the low activity of hepatic lipase excreted from the liver parenchym[18]. In addition, esterified cholesterol is frequently decreased in severe liver diseases, because the esterification of free cholesterol is disturbed by the lowered activity of the enzyme, that is LCAT[19].

The patients with chronic liver diseases in the present examination produced almost the same results as above and in addition to them, serum phospholipids also showed a significant change in some of these liver diseases (Table 2).

The recent advancement of the lipid researches discovered that HDL also changes in various hepatobiliary diseases. Vogt et al.[20] noted that seurm α-lipoprotein (HDL) was low in patients with acute hepatitis, chronic active hepatitis and liver cirrhosis.

It was also proven in the animal experiment with rats treated with praceodymiun by Lehmann et al.[21] that HDL lipids can be an important and excellent indicator for the liver function test.

The d.$>$1.063 franction examined in the present study contains not only HDL but also VHDL. However, because the majority of cholesterol, triglycerides and apo-A belongs to the HDL fraction alone and phospholipids belong the both HDL and VHDL (phospholipids in VHDL is mainly lysolecithin)[18], HDL-cholesterol, triglycerides and apo-A correspond to those in d.$>$1.063 fraction with an exception of HDL-phospholipids.

When the patients with chronic aggressive hepatitis and liver cirrhosis were divided for comparison in this experiment into 2 groups each, i. e., 2A and 2B in the former and compensated and decompensated stages in the latter respectively, many chronic liver diseases, particularly in the advanced and severe stages, revealed the low levels of HDL-cholesterol and apo-A. Phospholipids of this fraction (d.$>$1.063) also decreased in these diseases, although there was statistical significance only in liver cirrhosis in the decompensated stage. On the contrary, HDL-triglycerides were higher in chronic aggressive hepatitis 2A and liver cirrhosis in the compensated stage.

The determination of lipids and apo-A in d.$>$1.063 fraction is, therefore, considered beneficial for the clinical examination and researches of these diseases.

HDL is classified into two groups in the serum according to the density, i. e., and HDL_2 and HDL_3. Their molecular weight and diameter of the particles are said to be quite different from each other[22].

As shown in Tables 4 and 5 in which HDL_2 and HDL_3 are compared, the decrease in cholesterol and apo-A in HDL_3 exceeds the decrease of those in HDL_2, especially in liver cirrhosis in the compensated and decompensated stages.

These results entirely differ from those seen in atherosclerotic patients in whom the decreased HDL level is primarily due to the decreased HDL_2 level[7,8]. These results further indicate that HDL_3 more represents the dysfunction of the liver than HDL_2 in these patients, although a slight decrease was observed in many constituents of HDL_2 fraction without statsitical significance except apo-A.

Furthermore, the decrease of phospholipids in VHDL in these diseases in the advanced stage indicates that the decrease of phospholipids in d.$>$1.063 fraction is due to the decrease of phospholipids in HDL_3 and VHDL.

According to the experiment of Patch et al.[10] incubation of HDL_3 particles mixed with triglyceride-rich lipoprotein in lipoprotein lipase produces the HDL_2-like particles. It is speculated, therefore, that HDL_3 was converted to HDL_2 receiving the constituents from surface of triglyceride-rich lipoprotein in the metabolic pathway in animal and human sera. Therfore, the HDL level is inversely correlated with the serum triglyceride level and low HDL_2 level is often accompanied by high triglyceride level in atherosclerotic patients[23,24].

The decline of HDL_2 level may be caused by the hepatic dysfunction, because, as described above, the HDL_2 level depends upon the degradation rate of triglyceride-rich lipoprotein which is also regulated by hepatic lipase, and hepatic lipase may be inhibited by the liver damage. But the absence of inverse correlation between HDL-cholesterol (d.$>$1.063) and serum triglyceride and the moderateness of magnitude of the decrease in HDL_2 observed in our present

study can hardly support that the inhibited hepatic lipase activity contributed much to the decrease in the HDL level of these level of HDL_3 seems to represent the decreased hepatic synthesis of HDL_3 and/or its excretion into the blood stream.

The considerably reduced LCAT activity was observed in these patients, especially in liver cirrhosis in the decompensated stage. During the reaction of this enzyme, lysolecithin, a product of this reaction, is carried by VHDL particles[25]. In view of the majority of phospholipids in VHDL being lysolecithin, it is possible to estimate that lowered phospholipids in VHDL seen in chronic liver diseases is due to the decreased activity of LCAT. However, there was no correlation between the levels of phospholipids in VHDL and LCAT activity. It is, therefore, considered that other factor(s) of the liver than LCAT reaction is involved in the reduction of phospholipids in VHDL and that the determination of phospholipids in VHDL may prove useful as a liver function test.

REFERENCES

1) Radding, C. H., Bragdon, L. H. and Steinberg, D.: The synthesis of low—and high-density lipoproteins in rat liver in vitro. Biochym. Biophys. Acta, 30, 443-444, 1958.

2) Johansson, C., Rössner, S. and Walldius, G.: HDL secretion from human intestinal tract. Lancet, I, 324-325, 1978.

3) Tall, A. R. and Small, D. M.: Plasma high-density lipoproteins. New Engl. J. Med., 299, 1232-1236, 1978.

4) Hamilton, R. C., Williams, M. C., Fielding, C. J. and Habel, R. J.: Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. J. Clin. Invest., 58, 667-680, 1976.

5) Miller, G. J. and Miller, N. E.: Plasma high density lipoprotein concentration and development of ischemic heart disease. Lancet, I, 16-19, 1975.

6) Kajiyama, G., Mizuno, T., Matsuura, C., Yamada, K., Suzukawa, M., Fujiyama, M. and Miyoshi, A.: The lowered serum phospholipids in α-lipoprotein in patients with atherosclerosis. Hiroshima J. Med. Sci., 23, 229-236, 1974.

7) Anderson, D. W., Nicholas, A. V., Pan, S. S. and Lindgren, F. T.: High density lipoprotein distribution. Resolution of determination of three major components in a normal population sample. Atherosclerosis, 29, 161-179, 1978.

8) Nakamura, H.: Prophylactic approach to hypertensive diseases. p. 491 Ed. Yamori, Y. (Perspectives in cardiovascular research Vol. 4) Raven Press, 1979.

9) Gofman, J. W., de Lalla, O., Glazier, F., Freeman, N. K., Lindgren, F. T., Nicholas, A. V., Strisower, B. and Tamplin, A. R.: Plasma, 2, 413-484, 1954.

10) Patch, J. R., Gotto, A. M., Olivercrona, T. and Eisenberg, S.: Formation of high density lipoprotein$_2$-like particles during lipolysis of very low density lipoproteins in vitro. Proc. Natl. Acad. Sci., 75, 4519-4523, 1978.

11) De Groote, J., Desmet, V. J., Gedigk, P., Korb, G., Popper, H., Poulsen, H., Schever, P. J., Schmid, M., Fhaler, H. and Vehlinger, E.: A classification of chronic hepatitis. Lancet, II, 626-628.

12) Burnstein, M., Scholnick, H. R. and Morfin, R.: Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res., 11, 583-595, 1970.

13) Kajiyama, G., Nakagawa, M., Takata, K., Nakao, S. and Miyoshi, A.: Determination of cholesterol and phospholipids in HDL_2, HDL_3 and VHDL in patients with atherosclerosis and diabetes mellitus. Hiroshima J.Med. Sci., 30, 175-182, 1981.

14) Allain, C. C., Poon, L. S. and Chan, C. S.: Enzymatic determination of total serum cholesterol. Clin. Chem., 20, 470-475, 1974.

15) Fletcher, M. J.: A colorimetric method for estimating serum triglycerides. Clin. Chim. Acta, 22, 393-397, 1968.

16) Takayama, S., Ito, S., Mori, A., Nagasaki, H., Tanimizu, K., Horiuchi, Y., Imamura, S. and Ikuta, S.: Enzyme method for the determination of choline. Japanese J. Clin. Path., 24 (Supple), 461, 1976. (Abst).

17) Nagasaki, T. and Akanuma, Y.: A new colourimetric method for the determination of plasma lecithincholesterol acyltransferase activity. Clin. Chim. Acta, 75, 371-375, 1979.

18) Klose, G., Windelband, J., Weizel, A. and Greten, H.: Secondary hypertriglyceridemia in patients with parenchymal liver disease. Europ. J. Clin. Invest., 7, 557-562, 1977.

19) Glomset, J. A.: The plasma lecithin: cholesterol acyltransferase reaction. J. Lipid Res., 9, 155-167, 1968.

20) Vogt, N. and Engelhardt, A.: Das Lipoproteinmuster und chronischer Leberkrankheitein. Dtsch. Med· Wöchenschr., 100, 2156-2160, 1975.

21) Lehmann, B., Crajewski, O., Arntz, H-R. and Oberdisse, E.: Correlation between serum high density lipoprotein content and liver function during experimental hepatic degradation and regeneration. Acta Hapto-Gastroenterol, 24, 328-333, 1976.

22) Levy, R. I., Blum, C. B. and Schaefer, E. J.: The composition, structure and matabolism of high density lipoprotein, pp. 56-64, Ed. Greten H. Lipoprotein metabolism. Springer-Verlag, Berlin Heiderberg, New York, 1976.

23) Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. P. and Dawber, T. R.: High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am. J. Med., 62, 707-714, 1977.
24) Tall, A. R. and Small D. M.: Plasma high density lipoproteins. New Engl. J. Med., 299, 1232-1236, 1978.
25) Switzer, S. and Eder, H. A.: Transport of lysolecithin by albumin in human and rat plasma. J. Lipid Res., 6, 506-510, 1965.