HDL AND ITS SUBFRACTIONS IN PATIENTS WITH
CHRONIC LIVER DISEASES*1

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
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ABSTRACT

Serum lipids, lipids (cholesterol, triglycerides and phospholipids) and apo-A in d.>1.063
fraction, HDL₂, HDL₃ and VHDL of 10 normal subjects and 34 patients with chronic
liver diseases (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 (HDL₂, HDL₃ and VHDL) was achieved
by relatively simple procedures with combination of precipitation method and ultra­
centrifugal 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 HDL₂ and HDL₃ tended to decrease but the magnitude of
their decrease in HDL₃ exceeded that in HDL₂. 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 HDL₃, 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 HDL₂ 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*² and intestine*³
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*⁴.

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

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*² 椎山哲朗，高田耕史，細内 重，小山田健，三好秋馬：慢性肝疾患の HDL およびその論分画
serum HDL level.

HDL is found in the discoidal nascent forms in the incubation medium after liver reflux of rats and in two different spheric forms (HDL₂ and HDL₃) in the blood stream.

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

The decrease in HDL₂ is significant when compared with the decrease in HDL₃ in these atherosclerotic patients and the decrease in HDL₂ 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 and, in addition, the HDL₂-like particles are produced in the incubation mixture of HDL₃ and triglyceride-rich lipoproteins.

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 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 biopsy and in some cases with laparoscopy.

The d>1.063 fraction which contains the HDL₂, HDL₃ and VHDL subfractions was separated by precipitating d<1.063 fraction according to the Burnstein's method. Fractionation of HDL₂, HDL₃ and VHDL was performed as described previously.

To be more concrete, after low and very low density lipoproteins (d<1.063) were precipitated by adding 4% sodium phosphotangstate and 2M MgCl₂ to serum, ultracentrifugation of the supernate was repeated twice with adjustment of density each time.

Cholesterol, triglycerides and phospholipids 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 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 contrary, 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

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

* 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

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Apo-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>55.9±12.2</td>
<td>16.8±4.5</td>
<td>102.8±16.6</td>
<td>185±22</td>
</tr>
<tr>
<td>CAH (2A)</td>
<td>52.1±12.9</td>
<td>24.3±4.9**</td>
<td>101.5±17.1</td>
<td>174±33</td>
</tr>
<tr>
<td>CAH (2B)</td>
<td>41.9±7.1**</td>
<td>22.0±7.6</td>
<td>92.0±11.7</td>
<td>149±16**</td>
</tr>
<tr>
<td>LC (Comp)</td>
<td>39.9±10.2**</td>
<td>28.4±10.2**</td>
<td>88.4±18.9</td>
<td>110±37***</td>
</tr>
<tr>
<td>LC (Decomp)</td>
<td>29.8±11.1**</td>
<td>22.7±9.2</td>
<td>62.8±17.7***</td>
<td>118±51*</td>
</tr>
</tbody>
</table>

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

Table 3. Lipid and Apo-A concentrations in HDL₄ (1.063<d.<1.125) fraction in normal subjects and patients with chronic liver diseases

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Apo-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>16.9±8.5</td>
<td>29.6±15.3</td>
<td>53.0±16.7</td>
</tr>
<tr>
<td>CAH (2A)</td>
<td>16.9±7.4</td>
<td>23.0±8.9</td>
<td>47.6±18.7</td>
</tr>
<tr>
<td>CAH (2B)</td>
<td>11.8±3.6</td>
<td>19.4±6.4</td>
<td>42.8±10.3</td>
</tr>
<tr>
<td>LC (Comp)</td>
<td>17.7±11.3</td>
<td>30.6±18.0</td>
<td>35.4±21.2</td>
</tr>
<tr>
<td>LC (Decomp)</td>
<td>11.5±4.9</td>
<td>19.8±7.6</td>
<td>23.1±10.2**</td>
</tr>
</tbody>
</table>

** p<0.01 statistically significant against normal subjects

Table 4. Lipid and Apo-A concentrations in HDL₅ (1.125<d.<1.210) fraction in normal subjects and patients with chronic liver diseases

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Apo-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>37.3±11.2</td>
<td>62.5±18.8</td>
<td>122.8±51.2</td>
</tr>
<tr>
<td>CAH (2A)</td>
<td>35.2±8.9</td>
<td>53.3±10.5</td>
<td>125.0±19.6</td>
</tr>
<tr>
<td>CAH (2B)</td>
<td>30.9±9.7</td>
<td>53.7±12.8</td>
<td>104.9±15.6</td>
</tr>
<tr>
<td>LC (Comp)</td>
<td>23.9±7.8**</td>
<td>47.0±15.0*</td>
<td>74.7±28.8*</td>
</tr>
<tr>
<td>LC (Decomp)</td>
<td>19.4±6.5**</td>
<td>37.5±9.3**</td>
<td>74.1±28.5*</td>
</tr>
</tbody>
</table>

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

CAH: Chronic active hepatitis, LC: Liver cirrhosis, Comp.: Compensated, Decomp.: Decompensated.

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
(1.25<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), phospho­
lipid (p<0.05 and p<0.01) and apo-A(p<0.05)
concentrations in compensated and decompen­
sated 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

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

| Normal subjects | 20.8±6.3 |
| CAH (2A) | 22.5±5.8 |
| CAH (2B) | 14.9±5.3* |
| LC (Comp) | 16.9±9.6 |
| LC (Decomp) | 12.0±6.0* |

*P>0.05 Statistically significant against normal subjects

significance in the correlation between serum
triglycerides and cholesterol in d.>1.063 frac­
tion in 10 normal subjects and 34 patients with
chronic liver diseases. (Fig. 1)

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

DISCUSSION
Serum lipids are known to alter in various
acute and chronic hepatobiliary diseases and
have been frequently adopted as a clinical indicator of hepatobiliary 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 parenchyma\(^{(18)}\). 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 serum \(\alpha\)-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 praceodymium by Lehmann et al.\(^{(21)}\) that HDL lipids can be an important and excellent indicator for the liver function test.

The d.\(>1.063\) fraction 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)\(^{(22)}\), 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\(^{(23)}\).

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\(_4\) more represents the dysfunction of the liver than HDL\(_2\) in these patients, although a slight decrease was observed in many constituents of HDL\(_4\) fraction without statistical 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\(_4\) and VHDL.

According to the experiment of Patch et al.\(^{(10)}\) incubation of HDL\(_4\) particles mixed with triglyceride-rich lipoprotein in lipoprotein lipase produces the HDL\(_2\)-like particles. It is speculated, therefore, that HDL\(_4\) was converted to HDL\(_2\) receiving the constituents from surface of triglyceride-rich lipoprotein in the metabolic pathway in animal and human sera. Therefore, the HDL level is inversely correlated with the serum triglyceride level and low HDL\(_4\) level is often accompanied by high triglyceride level in atherosclerotic patients\(^{(25,34)}\).

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 HDL₃ seems to represent the decreased hepatic synthesis of HDL₃ 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. 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

