Characterization of Alterations in Plasma Lipoprotein Lipid and Apoprotein Profiles Accompanying Hepatoma-induced Hyperlipidemia in Rats

Ronald W. Clark and Richard C. Crain

Department of Molecular and Cell Biology, U-125, The University of Connecticut, Storrs, Connecticut 06268

ABSTRACT

Alterations in plasma lipoprotein lipid and apoprotein accompanying the hyperlipidemia of rats bearing Morris hepatoma 7288C were characterized. In tumor-bearing animals all plasma lipid classes except cholesterol ester (CE) were elevated, particularly free cholesterol (FC) and triglyceride (TG), which increased by 57 and 63%, respectively. Fasting only partially reduced the tumor-induced hyperlipidemia and had no effect on the ratios of FC/CE and TG/CE. Analysis of plasma lipoproteins revealed an elevation of VLDL, IDL, and LDL in host rats, with more than a 2-fold increase in both lipid and protein of VLDL. In contrast, the three high density fractions, HDL1, HDL2, and d > 1.21 g/ml, were reduced. The inverse changes in concentration of host lipoproteins of lower versus higher density indicate a defective catabolism of TG-rich lipoprotein. This possibility is supported by the analysis of apolipoprotein. The percentage of total apoprotein contributed by apo-C-I and C-II was reduced in all host fractions except HDL2, while the C-IIIIs remained unchanged except for a small decrease in C-III-3 of host VLDL and a slight increase in the combined C-IIIIs of HDL2. These changes were reflected in the decreased C-I+C-II/C-III ratios of all host lipoprotein fractions. Apo E levels remained similar to control values except for a significant decrease in HDL2. Host VLDL showed increased apo A-IV and A-I content, while A-IV was decreased in HDL2. Changes in apo A profiles were also observed.

INTRODUCTION

Plasma hyperlipidemia is a well documented phenomenon associated with tumor growth. During the development of Ehrlich ascites tumor in mice (1), Walker carcinosarcoma (2), mammary adenocarcinoma (3), and Morris hepatoma (4) in rats, and simian virus 40F tumor (5) in hamsters, pronounced cancer-induced hyperlipidemia has been noted. In humans, cancer of the liver (6, 7), breast (8, 9), ovary (9), and colon (10), as well as leukemia (8, 9) and lymphoma (9), have also been associated with elevated plasma lipid.

This increase in plasma lipid, moreover, is not simply due to a general rise of all lipoprotein classes. Barclay and Skipos (8) and Barclay et al. (9, 11) have reported that in both rats (11) and humans (8, 9) with cancer, the hyperlipidemia observed was characterized by an elevation of VLDL and a decrease in HDL. Similar alterations were found with SV40F tumors (5) and murine leukemia (12). A question receiving increasing attention is by what mechanism(s) is this specific type of hyperlipidemia produced.

Interestingly, noncancerous human subjects with high plasma VLDL have been shown to have decreased HDL concentrations (13). Furthermore, a significant positive correlation between plasma HDL cholesterol and LPL activity in adipose tissue has also been reported (14), suggesting that LPL activity and the resultant rate of catabolism of triglyceride rich lipoproteins might be an important factor in determining plasma HDL levels. In this context several recent reports are significant. Both Thompson et al. (15) and Lanza-Jacoby et al. (3) found that in tumor-bearing mice and rats, respectively, the earliest observed change was a decline in the activity of adipose tissue LPL. Likewise, Lyon et al. (16) found the clearance rate of labeled VLDL triglyceride reduced in mice with Ehrlich ascites tumor, and the cancer-induced hypertriglyceridemia occurred despite a decrease in the rate of hepatic triglyceride secretion. While lipase activities declined in these tumors rodents, endogenous lipogenic rates in both liver and adipose tissue also declined (3, 15, 17). Therefore, in these cases of hyperlipidemia, a defective catabolism rather than an elevated hepatic synthesis of lipoprotein seems to be the cause.

In the rat, both chylomicron and VLDL remnants are rapidly taken up by the liver, and in contrast to humans, little VLDL is converted to LDL (18). Serving as determinants of hepatic uptake of these remnant particles are various apoproteins. Apoprotein E appears to be the crucial marker for the high affinity binding by hepatic receptors, while the C apoproteins, particularly C-III, seem to oppose this recognition (19—22). Triglyceride-rich lipoproteins deficient in apo E (23) and/or elevated in apo C-III (24) have been associated with several types of hyperlipoproteinemia.

Apart from a favorable E/C-III ratio, the normal catabolism of chylomicrons and VLDL requires the obligatory cofactor of LPL, apoprotein C-II. Apo C-II has been shown to be a potent activator of LPL from a variety of sources (25—27). Apo C-I has also been shown to enhance LPL activity (28, 29). In contrast, apo C-III inhibits LPL (30, 26). Therefore the C-I+C-II/C-III ratio may act as an important regulator of the LPL catalyzed hydrolysis of lipoprotein triglyceride. Indeed, reduced C-II/C-III ratios have been implicated in primary and secondary hypertriglyceridemia (31, 32). Moreover, certain cases of familial type I hyperlipoproteinemia now appear to be caused by C-II deficiency (33, 34). Significant in this regard is a recent study in which the hypertriglyceridemia induced in mice by GRSL ascites tumor was associated with a decrease in LPL activity and a shift in the apo C pattern observed by SDS-PAGE (35). Although the individual C apoproteins were not identified, the gel band profiles indicate that plasma C-III increased with tumor growth, while C-II decreased.

During the present investigation, the distribution of lipid and apoprotein among all lipoprotein subfractions was examined. Our results show that hepatoma 7288C bearing rats exhibit elevated plasma VLDL and LDL and reduced HDL2. The apo C-I+C-II/C-III ratio was decreased in all lipoprotein fractions, especially LDL, in tumor-bearing rats, compared to controls, while the host E/C-III ratio was relatively unchanged in VLDL, LDL, and HDL2 and was decreased in HDL3. Changes in the proportion of the different apo B species found in VLDL and

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HDL1, high density lipoprotein subfraction 1; HDL2, high density lipoprotein subfraction 2; HDL3, high density lipoprotein subfraction 3; HTC, hepatoma tissue culture; FC, free cholesterol; CE, cholesterol ester; TG, triglyceride; LPL, lipoprotein lipase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PHP, post-heparin plasma.
LDL were also noted. In light of our recent finding that HTC cells, derived from 7288C tumors, secrete a nonspecific lipid transfer protein (36), and since our preliminary studies with hepatoma-bearing rats demonstrated significantly lowered hematocrits, the cellular component of whole blood was also examined to determine whether or not changes in blood cell lipid might accompany the cancer associated anemia. The lipid composition of host cells was found to be similar to that of controls, while the decreased phospholipid (24%) and free cholesterol (29%) found in the cellular compartment of blood can be explained by the similar decrease in hematocrit.

MATERIALS AND METHODS

Animals

Male Buffalo strain rats, 12–16 weeks old and weighing 250–325 g at the time of sacrifice, were utilized in all experiments. The animals were housed in community cages (2–4 rats/cage), exposed to a 12 h light-12 h dark cycle (light 6 a.m. to 6 p.m.), and allowed free access to Purina rat chow and water.

Morris hepatoma 7288C was obtained from Drs. Morris and Criss of the Cancer Research Unit of the Department of Biochemistry at Howard University College of Medicine. The hepatoma was maintained by bilateral s.c. implantation in the hind limbs with tumor cell homografts (approximately 20–25 × 10^6 cells/animal). Under these conditions the survival time of the tumor-bearing rats was 17–19 days.

Preparation of Blood and Plasma. For experiments involving fasted animals, food was removed 18–20 h before blood sampling. Tumor-bearing rats were sacrificed 13–15 days after implantation when the tumors had grown to 2.5–3.0 cm in diameter. At this point the combined wet weight of two tumors was 14–17 g and accounted for 5–6% of total body weight. Blood was collected between 10:30 a.m. and 2 p.m. For sampling, both control and tumor-bearing rats were anesthetized with pentobarbital, and blood (5–9 ml) was withdrawn from the abdominal aorta into a syringe containing 1 ml 50 mM EDTA (pH 7.8). The blood was then transferred to glass centrifuge tubes and processed according to the recommendations of Bachorik (37). Within 30 min of collection, the plasma was isolated by centrifugation at 2400 rpm for 30 min (35,000 × g-min) at 4°C. In all cases the hematocrit was recorded, and the plasma was transferred to fresh tubes and respun to pellet any remaining cells and debris. The isolated plasma was then dialyzed for 3–4 h against 150 mM NaCl:2 mM EDTA (pH 7.8):0.02% NaN3 (NaCl-EDTA buffer), after which aliquots were set aside on ice for later analysis. Where lipid analysis of both plasma and cellular compartments of the blood was desired, the pellets from the previous centrifugations (plus any remaining plasma associated with them) were combined and mixed well, and an aliquot was set aside. Analysis of this aliquot, combined with that of the isolated plasma, allowed total blood lipid to be calculated. The remainder of the cell-plasma mix was then washed twice in 40 ml of NaCl-EDTA buffer, centrifuging each time at 15,000 rpm for 25 min (675,000 × g-min) to isolate the cellular fraction of the blood.

Lipoprotein Fractionation. For studies requiring isolation of lipoprotein subclasses, plasma from 3–4 control or hepatoma-bearing rats was pooled before dialysis. After dialysis, 2.3 ml of plasma was added to each of five tubes containing solid KBr. To a sixth tube was added 2.1 ml of plasma plus 0.2 ml of Sudan black/dimethyl sulfoxide (0.2 mg/ml). This prestained tube allowed the subsequent separation of lipoproteins to be visualized (38). Solid KBr was brought into solution by gentle, repeated inversion, achieving a final density in all tubes of 1.250 g/ml at 20°C. Each adjusted plasma aliquot was transferred to a tube calibrated in 1.0-ml increments (tube capacity = 12 ml). Two KBr solutions, in 2 M EDTA (pH 7.8), were next layered successively into each tube: 3.0 ml of d = 1.120 g/ml and 3.0 ml of d = 1.050 g/ml. The density of these solutions was checked by refractometry. Finally, 3.2 ml of double distilled water was added, bringing the total volume to 11.7 ml/tube.

The six gradients were attached to a Beckman SW41Ti rotor and spun at 40,000 rpm for 23 h. After centrifugation the lipoprotein subfractions from the five unstained tubes were isolated by tube slicing utilizing the sixth stained gradient as a visual guide. The six plasma subclasses obtained constituted the whole plasma. These were: VLDL, \( d < 1.006 \) g/ml; LDL, \( d = 1.006-1.202 \) g/ml; LDL, \( d = 1.020-1.050 \) g/ml; HDL, \( d = 1.050-1.120 \) g/ml; HDL, \( d = 1.120-2.120 \); and all material of \( d > 1.210 \) g/ml including very high density lipoprotein and albumin. All plasma fractions were dialyzed extensively against NaCl-EDTA buffer and then stored on ice in a closed container until analysis.

Analytical Procedures. Plasma and lipoprotein total lipid was extracted by a modification of the method of Bligh and Dyer (39). For blood cell fractions it was necessary to extract by the method of Rose and Oklander (40), since the Bligh and Dyer procedure extracts a protein that interferes with subsequent spectrophotometric assays. Studies performed in our laboratory showed the extraction of plasma by either technique gave the same values for phospholipid, cholesterol, and triglyceride content.

Lipid phosphorus (41) and protein concentrations (42) were determined by standard assays. Total and free cholesterol were quantified by an enzymatic method (43, 44) in the presence and absence of cholesterol esterase, respectively. Cholesterol ester was calculated by the difference between total and free cholesterol. Triglyceride was determined by a modification of published methods (45, 46), which allowed quantitation of samples in the range of 10–100 nmol.

Electrophoresis. In order to prevent distortion by lipid during electrophoresis, VLDL and LDL fractions were first delipidated. The delipidation procedure used was a modification of that developed by Scanu and Edelstein (47). Comparison of delipidated and undelipidated HDL2 by electrophoresis gave identical results, both in terms of the number of resolvable bands and the relative quantities. Thus it has been assumed that no apoprotein was lost upon delipidating VLDL and LDL. It was necessary however to use freshly redistilled diethyl ether in this delipidation procedure. Failure to follow this precaution led to the production of spurious electrophoretic bands. To evaluate the apoprotein composition of the four major lipoprotein subclasses of \( d < 1.210 \) g/ml, SDS-PAGE using both high and low percentage gels was performed. For resolution of proteins in the molecular weight range of 7,000–90,000, 15% acrylamide-18% glycerol tube gels were utilized according to the method of Connolly and Kukas (48). To these gels were applied 35–45 μg of one of the following lipoproteins: delipidated VLDL or LDL or undelipidated HDL2 or HDL3. Electrophoresis was carried out at a constant current of 5.0 mamp/gel for 18–19 h. For resolution of larger proteins, including the B apoproteins, 4.2% acrylamide-3% glycerol gels were formed. Twenty to 35 μg of protein were applied and electrophoresis performed at 4.5 mamp/gel for 14–15 h. All lipoprotein samples were reduced with dithiothreitol before electrophoresis, and the running buffer used for both high and low percentage gels was that of Weber and Osborn (49). After staining and destaining, gels were scanned using a Biomed Instruments densitometer (model SL-504-XL). Because the chromogenicity of different apoproteins may vary, densitometry is inadequate for an accurate determination of protein mass distribution. However the chromogenicity of the same apoproteins from different plasma samples should be the same. Therefore, densitometry is sufficient for revealing differences in control versus host apolipoprotein profiles.

For protein molecular weight standards, either the Sigma Dalton Mark VII-L mix (7 proteins, \( M_w 14,200-66,000 \)) or the Bio-Rad low molecular weight mix (6 proteins, \( M_w 14,400-92,500 \)) were used for 15% gels. For 4.2% gels both the Sigma high molecular weight mix (6 proteins, \( M_w 29,000-205,000 \)) and cross-linked albumin mix (pentamer-monomer, \( M_w 66,000-330,000 \)) were used.

RESULTS

Plasma Lipid and Protein. All tumor-bearing animals were sacrificed 13–15 days after implantation. At this stage the rats exhibited plasma hyperlipidemia, while the tumors were not yet necrotic, nor were the rats in a moribund condition. The lipid and protein content of whole plasma collected from control and hepatoma-bearing rats is presented in Table 1. While plasma protein is decreased nearly 20% in the tumor-bearing animals,
HEPATOMA-INDUCED PLASMA HYPERLIPIDEMIA

<table>
<thead>
<tr>
<th></th>
<th>Total phospholipid</th>
<th>Free cholesterol</th>
<th>Cholesterol ester</th>
<th>Total cholesterol</th>
<th>Triglyceride</th>
<th>Total lipid</th>
<th>Protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (fed)</td>
<td>23</td>
<td>1083 ± 122 (36.4)</td>
<td>275 ± 114 (9.1)</td>
<td>599 ± 124 (20.2)</td>
<td>875 ± 178 (29.3)</td>
<td>1066 ± 34 (34.1)</td>
<td>3023 ± 550 (60.3)</td>
</tr>
<tr>
<td>Hepatoma (fed)</td>
<td>23</td>
<td>1286 ± 189 (32.2)</td>
<td>431 ± 116 (10.8)</td>
<td>612 ± 150 (15.6)</td>
<td>1044 ± 180 (26.5)</td>
<td>1738 ± 630 (41.4)</td>
<td>4068 ± 831 (49.1)</td>
</tr>
<tr>
<td>Hepatoma (fasted)</td>
<td>14</td>
<td>1211 ± 93 (33.0)</td>
<td>389 ± 62 (10.7)</td>
<td>534 ± 60 (14.6)</td>
<td>923 ± 117 (25.3)</td>
<td>1552 ± 357 (41.7)</td>
<td>3685 ± 355 (ND)</td>
</tr>
</tbody>
</table>

* n, number of rats (separate determinations).
* Total lipid values were determined from combined values of total phospholipid, cholesterol, and triglyceride.
* Mean ± SD.
* P < 0.0005 by unpaired Student's t-test compared to control plasma.
* P < 0.05.
* P < 0.05 compared to hepatoma (fed).
* ND, not determined.

The levels of all lipid classes, except cholesterol ester, are elevated, particularly free cholesterol and triglyceride (57 and 63% increase). While fasting partially reduces the tumor-induced hyperlipidemia (P < 0.05 for total cholesterol and cholesterol ester), it by no means eliminates it, and plasma lipid remains elevated over that of fed control rats. Furthermore, the FC/CE and TG/CE ratios of the fasted tumorous rats (0.73 and 2.91, respectively) are more similar to those of the fed tumorous rats (0.70 and 2.84) than to the controls (0.46 and 1.78). Other investigators have observed similar increases in plasma FC/CE and TG/CE ratios associated with tumor growth in mice (12) and rats (4).

**Lipoprotein Distribution.** Lipoprotein subfractions were separated by density gradient centrifugation. A typical stained gradient after centrifugation is shown in Fig. 1. The only observable difference between gradients containing host rat plasma or control plasma (tube shown) was that the HDL₂ fraction of the former was somewhat narrower and fainter. In all cases the observed bands were within the density boundaries set by tube slicing. Since very little HDL₁ (d = 1.050—1.063) was present, only one fraction was isolated from the density 1.050—1.120 interval, and this was assumed to consist mainly of HDL₂ and is labeled so in Tables 2 and 3.

In Table 2 the distribution of lipid classes and protein among the six plasma fractions is shown. The host plasma VLDL, IDL, and LDL are elevated, while the remaining fractions are reduced. The greatest change occurred in VLDL, where all lipid classes and protein more than doubled.

Table 3 demonstrates that in spite of large changes in the amounts of various fractions, the differences in lipid composition between control and host lipoproteins are slight. For host VLDL, phospholipid is slightly increased and triglyceride is decreased (P < 0.1), while in LDL more cholesterol is found in the esterified form. In contrast, the HDL₃ fraction reveals a significant drop in the proportion of cholesterol ester, while free cholesterol remains near control levels. Consequently, the FC/CE ratio is significantly decreased in host LDL but increased in host HDL₃.

Distribution of Whole Blood Lipid. The proportion of lipid found in the plasma versus the cellular compartment of whole blood is shown in Table 4 for fed animals. The major change seen in the blood of hepatoma-bearing rats is a decreased quantity (nmol/ml whole blood) of phospholipid and free cholesterol found in the cellular compartment (24 and 29% decrease, respectively). It appears that this reduction can be explained by the similar (24%) decrease in host hematocrit. Whether or not the reduced hematocrit is in part due to an increase in blood volume was not determined.

In control blood 78% of the phospholipid and 88% of the free cholesterol is cellular, while in the anemic host blood these values are reduced to 67 and 78%. In contrast, essentially all cholesterol ester and triglyceride is in the plasma compartment.
### Table 2 Plasma lipoprotein lipid and protein distribution

<table>
<thead>
<tr>
<th>Material of d &gt; 1.210 g/ml</th>
<th>Total plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phospholipid</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>5</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>5</td>
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<tr>
<td>Triglyceride</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>5</td>
</tr>
<tr>
<td>Total lipid</td>
<td></td>
</tr>
<tr>
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<table>
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<td></td>
</tr>
<tr>
<td>Hepatoma</td>
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</table>

<table>
<thead>
<tr>
<th>n*</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL₂</th>
<th>HDL₃</th>
<th>Material of d &gt; 1.210 g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/ml plasma (% total lipid class)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total phospholipid          |              |
| Control                    | 6            |
| Hepatoma                   | 5            |
| Free cholesterol           |              |
| Control                    | 5            |
| Hepatoma                   | 5            |
| Cholesterol ester          |              |
| Control                    | 5            |
| Hepatoma                   | 5            |
| Total cholesterol          |              |
| Control                    | 5            |
| Hepatoma                   | 5            |
| Triglyceride               |              |
| Control                    | 5            |
| Hepatoma                   | 5            |
| Total lipid                |              |
| Control                    | 5            |
| Hepatoma                   | 5            |

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<tr>
<td>Control</td>
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<tr>
<td>Hepatoma</td>
<td></td>
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</tbody>
</table>

in both control and host blood. Although a change in quantity of cellular lipid has occurred, no statistical difference was found in the composition of the host cells versus the control.

Low Molecular Weight Apolipoproteins. Proteins of apparent molecular weight 7,000–90,000 were resolved on 15% acrylamide gels. The apoprotein patterns of the four main lipoprotein fractions are shown for both control and host plasma in Fig. 2. Seven commonly recognized apolipoproteins of rat plasma are clearly evident for HDL₂: apo A-IV; E; A-I; C-III-3; C-III-0; C-II; and C-I. To a greater or lesser extent these apoproteins are also observed for the other three lipoprotein fractions. In addition, several other bands are seen. In HDL₂ two major protein bands of M₆, 66,000 and 52,000 are tentatively identified as albumin and immunoglobulin heavy chain. In gels of HDL₂ and HDL₃ two more bands, designated U-I and U-II, are also present. These two proteins were consistently seen in all gels of HDL and have been observed in rat HDL by other investigators (48). When electrophoresis was performed

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### Table 3 Lipid composition of plasma lipoproteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL₂</th>
<th>HDL₃</th>
<th>Material of d &gt; 1.210 g/ml</th>
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<tbody>
<tr>
<td></td>
<td>TPL⁺</td>
<td>FC⁺</td>
<td>CE</td>
<td>TC</td>
<td>TG</td>
<td>FC/CE</td>
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<tr>
<td>VLDL</td>
<td>Control</td>
<td>6</td>
<td>15.8 ± 2.5⁴</td>
<td>9.9 ± 2.0</td>
<td>2.4 ± 1.0</td>
<td>12.3 ± 4.2</td>
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<td></td>
<td>5</td>
<td>18.4 ± 2.7⁴</td>
<td>10.6 ± 1.3</td>
<td>3.2 ± 1.7</td>
<td>13.8 ± 1.5</td>
<td>67.7 ± 1.6</td>
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<tr>
<td>IDL</td>
<td>Control</td>
<td>6</td>
<td>21.5 ± 3.9</td>
<td>24.7 ± 6.9</td>
<td>11.4 ± 3.7</td>
<td>36.1 ± 8.3</td>
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<tr>
<td></td>
<td>5</td>
<td>23.2 ± 1.4</td>
<td>21.7 ± 3.7</td>
<td>13.4 ± 4.0</td>
<td>34.9 ± 2.5</td>
<td>41.8 ± 3.7</td>
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<tr>
<td>LDL</td>
<td>Control</td>
<td>6</td>
<td>27.2 ± 3.0</td>
<td>22.9 ± 2.4</td>
<td>36.4 ± 3.6</td>
<td>59.3 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>26.0 ± 1.6</td>
<td>18.7 ± 2.2</td>
<td>43.3 ± 3.6</td>
<td>62.0 ± 2.0</td>
<td>12.0 ± 3.3</td>
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<tr>
<td>HDL₂</td>
<td>Control</td>
<td>6</td>
<td>44.7 ± 3.2</td>
<td>14.5 ± 1.9</td>
<td>38.3 ± 1.9</td>
<td>52.7 ± 2.5</td>
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<tr>
<td></td>
<td>5</td>
<td>48.0 ± 1.5†</td>
<td>13.0 ± 2.4</td>
<td>36.6 ± 1.8</td>
<td>49.6 ± 1.2</td>
<td>2.5 ± 1.1</td>
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<td>HDL₃</td>
<td>Control</td>
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<td>40.6 ± 3.2</td>
<td>11.5 ± 2.4</td>
<td>39.5 ± 0.6</td>
<td>51.1 ± 6.8</td>
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<td>5</td>
<td>45.6 ± 3.2²</td>
<td>13.0 ± 2.1</td>
<td>32.0 ± 2.2</td>
<td>45.1 ± 3.3</td>
<td>9.4 ± 4.7</td>
</tr>
</tbody>
</table>

* n, number of experiments.
*⁺ Mean ± SD.
*⁻ P < 0.05 by unpaired Student's t-test.
*⁺⁺ P < 0.01.
*⁻⁻ ND, not determined.
**Table 4: Distribution of blood lipids**

<table>
<thead>
<tr>
<th></th>
<th>Total phospholipid</th>
<th>Free cholesterol</th>
<th>Cholesterol ester</th>
<th>Total cholesterol</th>
<th>Triglyceride</th>
<th>Total lipid</th>
<th>Hematocrit</th>
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<td><strong>Control</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plasma</td>
<td>14</td>
<td>686 ± 57 (21.8)</td>
<td>203 ± 38 (12.2)</td>
<td>375 ± 78 (99.0)</td>
<td>578 ± 80 (28.2)</td>
<td>570 ± 162 (93.0)</td>
<td>1834 ± 146 (31.6)</td>
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<tr>
<td>Cells</td>
<td>14</td>
<td>2457 ± 229 (78.2)</td>
<td>1465 ± 189 (87.8)</td>
<td>3.6 ± 6.1 (1.0)</td>
<td>1469 ± 187 (71.8)</td>
<td>43.2 ± 24.2 (7.0)</td>
<td>3969 ± 319 (68.4)</td>
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<td>Whole blood</td>
<td>14</td>
<td>3144 ± 260 (100.0)</td>
<td>1668 ± 195 (100.0)</td>
<td>379 ± 78 (100.0)</td>
<td>2047 ± 180 (100.0)</td>
<td>613 ± 170 (100)</td>
<td>5803 ± 368 (100.0)</td>
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<td><strong>Hepatoma</strong></td>
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<td></td>
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<td>Plasma</td>
<td>14</td>
<td>921 ± 99 (33.0)</td>
<td>300 ± 67 (22.3)</td>
<td>433 ± 98 (96.9)</td>
<td>733 ± 122 (40.9)</td>
<td>1249 ± 284 (96.6)</td>
<td>2902 ± 450 (49.4)</td>
</tr>
<tr>
<td>Cells</td>
<td>14</td>
<td>1873 ± 195 (67.0)</td>
<td>1047 ± 184 (77.7)</td>
<td>13.7 ± 17.7 (3.1)</td>
<td>1061 ± 183 (59.1)</td>
<td>44.3 ± 15.7 (3.4)</td>
<td>2978 ± 340 (50.6)</td>
</tr>
<tr>
<td>Whole blood</td>
<td>14</td>
<td>2797 ± 200 (100.0)</td>
<td>1347 ± 179 (100.0)</td>
<td>447 ± 106 (100.0)</td>
<td>1794 ± 202 (100.0)</td>
<td>1294 ± 290 (100.0)</td>
<td>5884 ± 436 (100.0)</td>
</tr>
</tbody>
</table>

*a*, number of fed rats (separate determinations).

*b* Mean ± SD.

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**Fig. 2. Resolution of rat plasma apolipoproteins (Mr, 7,000–90,000) by SDS-PAGE.**

**Lane S,** protein standards; **lanes a-d,** control VLDL, LDL, HDL2, and HDL3; **lanes e–h,** host VLDL, LDL, HDL2, and HDL3. The calculated apparent molecular weights of the major apoproteins were: C-I, 7,790 ± 120 (SD) (n = 20); C-II, 8,230 ± 120 (n = 21); C-III-0, 9,900 ± 230 (n = 30); C-III-3, 11,100 ± 300 (n = 30); U-II, 12,700 ± 200 (n = 24); A-I, 25,400 ± 600 (n = 36); E, 35,500 ± 1,100 (n = 38); and A-IV, 46,100 ± 1,200 (n = 27).

on lipoprotein samples without prior treatment with dithiothreitol, neither U-I nor the M, 52,000 band were evident (gels not shown). U-I, M, 22,000, is therefore tentatively identified as immunoglobulin light chain. A protein of the same molecular weight was also apparent in VLDL and occasionally detectable in LDL. U-II, on the other hand, was evident only in HDL2 and HDL3, had an apparent molecular weight of 12,700, and was present with and without dithiothreitol treatment. As seen in Fig. 3, U-II is unaffected by sialidase treatment, where C-III-3, a sialylated form of C-III-0, disappears with a concomitant increase in C-III-0. This demonstrates that U-II is not a sialylated form of C-III.

The proportions of the apoproteins found in control and host VLDL, LDL, HDL2, and HDL3 are shown in Table 5. Where resolution between C-I and C-II was insufficient for quantitation by densitometry, only the combined C-I+C-II value is given. The percentage of C-I+C-II is reduced in all host fractions except HDL2, while the C-IIIIs remain unchanged except for a small decrease in the C-III-3 of VLDL and a slight increase in the combined C-IIIIs of HDL2. These changes are reflected in the decreased C-I+C-II/C-III ratios of all fractions listed in Table 6. In host VLDL, a significant increase in the proportion of apo A-IV and A-I occurs, while a decrease in A-IV is observed in the HDL2 fraction. The percentage of Apo E remained similar to control values except for HDL2, in which there was a rather large decline resulting in a corresponding decrease in the E/C-III ratio of this fraction (Table 2). Protein bands of molecular weight 75,000–90,000 were inconsistently seen in both control and host gels and do not appear to represent real differences between the two.

High Molecular Weight Apoproteins. Proteins of apparent molecular weight 25,000–500,000 were resolved on 4.2% acrylamide gels. In Fig. 4 the high molecular weight proteins consistently seen in VLDL and LDL fractions are pictured. The apparent molecular weights of these three apo B species were calculated to be 336,000, 318,000, and 214,000 and are designated B-336, B-318, and B-214. These apoproteins correspond to the P1, P11, and P111 (smallest) rat B apolipoproteins described by other investigators (50). In both host and control HDL2 these apoproteins, when measurable, represented less than 1% of the total protein. No apo B protein was detected in the HDL3 fraction (gels not shown). In addition to apo B species, apo A-III and A-I were also resolved on the low percentage gels. The resolution of apo E on both high and low percentage gels allowed the construction of Table 7, where the proportions of the three apo Bs are expressed as their relative contribution to the total apolipoprotein (M, 7,000–500,000). For control and host fractions total apo B accounts for 13.2
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Fig. 3. Effect of sialidase treatment on control plasma HDL₂ apolipoproteins as determined by SDS-PAGE. Lane a, without sialidase treatment; lane b, after sialidase treatment. Desialylation was achieved by combining 4.5 μg sialidase (Sigma Chemical Co.) per mg HDL₂ protein in 100 mM sodium acetate (pH 5.0) and incubating at 37°C for 90 min.

and 18.7%, respectively, of total VLDL apoprotein and 32.5% and 39.4%, respectively, of total LDL apoprotein. Besides the increased fraction of apo Bs in host VLDL and LDL, a change in proportion of the different apo B species is observed. As shown in Table 3, B-214 is the major apo B species in control VLDL, while the B-336+318 species predominate in control LDL. In host VLDL and LDL these proportions are altered; the B-214/B-336+318 ratio declines from 1.53 to 1.16 in the former and rises from 0.13 to 0.27 in the latter.

Tables 5–7 demonstrate that, unlike human LDL, rat LDL is a heterogeneous fraction containing not only apo B but other apoproteins such as E and several apo Cs. Such heterogeneity has been observed by other investigators, even for the narrower density cut of d = 1.030–1.050 g/ml (51, 52). A similar variety of apoproteins is also seen in mouse LDL (35). The presence of these additional apoproteins may be explained by the presence of VLDL-like particles which, whether partially catabolized or not, possess densities that overlap into the low density region.

DISCUSSION

Plasma hyperlipidemia and the depletion of lipid from fat stores are frequently associated with cancer in both experimental animals (2–4, 53) and in humans (6, 9). The tumor-induced hyperlipidemia develops independently of dietary fat (54), although total fasting does reduce its degree (1), a result confirmed in the present study. A dietary caloric source, therefore, appears to be involved. Depletion of carcass lipid (55), on the
was accompanied by a slight increase in heart LPL activity and the appearance of substantial activity in large tumors. The authors of the former report have proposed that these alterations could be responsible for a redistribution of lipid away from storage in the adipose tissues to the other host tissues and, as the tumor grows, to the tumor itself.

In the present report we have characterized the plasma and blood lipid distributions of rats bearing Morris hepatoma 7288C. All plasma lipid classes were elevated, except cholesterol, and a corresponding increase in FC CE and TG CE ratios, compared to control, was described. This hyperlipidemia was distinguished by an increase in plasma VLDL, IDL, and LDL, and a decrease in the 3 high density fractions. Similar alterations in the plasma lipoprotein profile have been observed both in human cancer patients (8, 9) and in animals bearing nonhepatic tumors (5, 11, 12), and this suggests a defective catabolism of TG-rich lipoprotein (14). In contrast, Narayan has reported that during carcinogenesis of the liver, serum catabolism of TG-rich lipoprotein (14). In contrast, Narayan, might be explained by the ability of both primary and trans B-336 — explained solely on nutritional grounds. More recently, Thomp...
appears to be particularly important for chylomicron catabolism, and differential reductions in the activities of LPL, C1 and LPL, C11, have been implicated in the etiology of several primary hyperlipoproteinemias (29, 65). Whereas apo C-I and C-II serve as cofactors of LPL, apo C-III acts as an inhibitor (26, 30). Therefore, the C1+1+C-11/C-III ratio may be an important regulator of the LPL catalyzed hydrolysis of triglyceride-rich lipoproteins, and reductions in this ratio have been associated with both primary and secondary hypertriglyceridemia (31, 32). In the present investigation, a decline in both C-I and C-II contributed to the reduction in this ratio for host VLDL. Lack of sufficient resolution between C-I and C-II in host LDL prevented individual quantitation of the two apoproteins. However, the host C1+C-11/C-III ratio did decrease to one-half that of control LDL. These altered ratios might reduce LPL activity, and an inhibition of LPL could explain the observed hyperlipidemia. Whether or not the altered apo C content observed does in fact affect plasma lipase activities is a matter of conjecture until enzymatic studies are conducted. Relevant to these findings is the recent study by Damen et al. (35), in which mice were observed following transplantation of GRSL ascites tumor. The first change noted was a decrease in LPL activity on day 2, and on day 3 hypertriglyceridemia was evident. However, when LPL was assayed in the presence of heat-inactivated control plasma, a decrease was not observed before day 5. This suggested the depletion of a cofactor preceding the final disappearance of the enzyme itself. Indeed, SDS-PAGE indicated a progressive decrease in the C1+C-11/C-III ratio after 2 days (35).

Apo E appears to be the determinant for the high affinity binding of chylomicron and VLDL remnants by hepatic receptors, while the C apoproteins, particularly C-III, seem to oppose this recognition (19–22). In this study, the host E/C-III ratio, compared to control values, was found to be relatively unchanged in VLDL, LDL, and HDL, but reduced significantly in LDL. The host VLDL and LDL ratios demonstrate that the tumor-induced hyperlipidemia is not caused by a lack of apo E. The relative apo E content in host LDL was increased slightly compared to control, although due to the variability of apo E in control LDL this difference was of low statistical significance (P < 0.20). In contrast, there was a marked reduction of E in HDL. Rat apo E has been shown to exchange between VLDL and HDL (66), and in humans Blum has found a strong correlation between plasma triglyceride concentrations and the fraction of apo E in triglyceride-rich lipoproteins (67). Therefore, the increase in apo E seen in host VLDL and decrease seen in HDL, might be due to a decrease in VLDL catabolism and, consequently, a lack of apo E transfer to HDL, although a reduction in the hepatic secretion of apo E-rich HDL might also contribute to the decreased E levels of host HDL. A reduced catabolism might also explain the increase of apo A-IV and A-I in host VLDL, since the LPL catalyzed lipolysis normally brings about a transfer of A-IV and A-I from chylomicrons and VLDL to the HDL fraction.

Alterations of apoprotein B content of host lipoproteins were also noted in this study. Normally, all three apo Bs are secreted by rat liver (68, 69) and hepatocytes in culture (70). The intestine, however, produces mainly B-214 (71). Both the synthesis of liver (69, 70) and intestinal (71) B-214 appears to be correlated with triglyceride synthesis, and this apo B species predominates in the TG-rich lipoproteins, VLDL, and chylomicrons. The B-336+318 doublet, on the other hand, constitutes the major apo B of LDL and serves as a determinant for receptor recognition of LDL by extrahepatic (72) and hepatic (73, 74) cells. The most significant change observed in host apoprotein B pattern is the increased B-214/B-336+318 ratio of LDL. This alteration is compatible with the reduced catabolism of triglyceride-rich lipoprotein proposed in this report. A buildup of partially hydrolyzed remnants would be expected to result in a greater B-214 concentration compared to B-336+318. The decreased ratio of host VLDL, on the other hand, may be due in part to a reduction in TG secretion by the liver.

An obvious question posed by tumor-induced hyperlipidemia is whether this condition is exploited by the developing tumor. Beirne and Watson have demonstrated that although HTC cells in culture exhibit feedback control of sterol synthesis, these same cells, when transplanted back into rats and grown as the 7288C hepatoma, fail to express such control (75). This difference in transplantable hepatomas compared to cultured hepatoma cells might be explained by an intrinsic difference in the way transplanted hepatomas react to blood lipid and/or the reduced blood supply of these tumors (76, 77). Both possibilities have been substantiated in recent studies. Barnard et al. (78) has shown that the binding of chylomicron remnants to 7288C hepatoma cell membranes is decreased 70–80% compared to liver membranes, whereas work by Redgrave et al. (79) suggests that the preferential uptake of chylomicron remnants by the liver might not be due to cellular specificity but to the lower capillary permeability of extra-hepatic sites compared to the liver sinusoid.

It is well documented that a cholesterol supply is required during cell growth (80, 81). A tumor might supply this demand solely by endogenous synthesis, and for HTC cells the synthesis of cholesterol from acetate has been demonstrated (78, 82). However, it would be advantageous for the tumor to avail itself
of plasma cholesterol and other lipid. But how might it compete
with the liver, which is endowed with a much greater blood
supply and a sinusoidal lining enriched in remnant receptors?
The answer might be to prevent the normal catabolism of
chylomicrons and VLDL and thus inhibit the receptor-medi-
ated uptake of plasma lipid. The relative importance of receptor-
independent uptake would be increased, allowing the tumor
to compete more equally.

Fielding et al. (83) have demonstrated that when cultured
fibroblasts are incubated with medium containing hyperli-
demic plasma (elevated VLDL and LDL), a net flux of chole-
stol from plasma to the cells occurs, whereas in incubations
with normolipidemic plasma, the net sterol transfer is from
cells to the plasma. The uptake of cholesterol in the former
case appears to be mediated by a nonreceptor mechanism
that is capable of effectively competing more equally.

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ted uptake of plasma lipid. The relative importance of receptor
independent uptake would be increased, allowing the tumor
to compete more equally.


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Characterization of Alterations in Plasma Lipoprotein Lipid and Apoprotein Profiles Accompanying Hepatoma-induced Hyperlipidemia in Rats

Ronald W. Clark and Richard C. Crain