Cholesterol Ester Fatty Acids in Serum and Liver of Normal and Lymph-fistula Rats*

LEON SWELL, M. D. LAW, HENRY FIELD, JR., AND C. R. TREADWELL

From the Veterans Administration Center, Martinsburg, West Virginia, and the Department of Biochemistry, School of Medicine, The George Washington University, Washington, D. C.

(Received for publication, February 12, 1960)

Data on the normal distribution of fatty acids in the cholesterol ester fraction of rat plasma and liver and on factors which may influence their distribution are extremely limited at the present time. The information available has been obtained by alkali isomerization and silicic acid and paper chromatography techniques. These methods do not provide an adequate identification and estimation of the individual fatty acid components. Mukherjee et al. (1) reported that in normal rat blood, although the cholesterol ester fraction had a higher iodine value than the other lipid fractions, saturated fatty acids made up 56% of the cholesterol ester fatty acids and that oleic acid was absent. Contrary to these findings, Klein and Janssen (2) reported that oleic acid constituted up to 59% of the cholesterol ester fatty acids of rat plasma. Studies (3-5) on the composition of the liver cholesterol ester fatty acids are in general agreement that this tissue contains a large proportion of saturated fatty acids and that these cholesterol esters differ markedly in composition from plasma cholesterol esters (3).

In an earlier study from our laboratories (6) with the aid of gas-liquid chromatography, it was shown that human serum and liver were also markedly different in their cholesterol ester fatty acid composition. The principal fatty acids of liver esters were found to be oleic and palmitic, whereas in the serum the polyunsaturated fatty acids constituted up to 52%, with linoleic acid the major fatty acid. These findings were in agreement with earlier studies (7, 8) which suggested that under normal conditions a large portion of the plasma cholesterol esters in the rat and man arise from extrahepatic sources. The present study was undertaken to provide quantitative data on the cholesterol ester fatty acid composition of normal rat serum and liver and to determine what effect the diversion of lymph cholesterol would have on the cholesterol ester fatty acid spectrum. Silicic acid chromatography followed by gas liquid chromatography were used to separate and quantitate individual fatty acid components.

EXPERIMENTAL PROCEDURE

Treatment of Animals and Tissues—The experimental lymph-fistula animals were adult male rats, weighing 225 to 250 g, of the Carworth strain; they had been maintained on Purina pellet chow. The preparation and care of the thoracic lymph-fistula animals have been described (7). After the operation six animals were given 0.9% sodium chloride solution to drink (no food) and killed 48 hours after the operation. A control group of five males was killed after 48 hours without food. At the time the animals were killed, blood (from the abdominal aorta) and the liver were removed. Lipid extracts of the liver and serum of each animal were prepared according to procedures described earlier (7, 9).

Methods—Free and total cholesterol were determined colorimetrically on the lipid extracts by the method of Sperry and Webb (10). Cholesterol esters were separated from the other lipid components by chromatography on silicic acid (11). Quantitative recovery of the sterol esters from the silicic acid column in the 1% ethyl ether-petroleum ether fraction was verified with the aid of labeled cholesterol-4-C\textsuperscript{14} olate and no overlap with the triglyceride fraction was noted as checked with triolein-1-C\textsuperscript{14}. The isolated cholesterol esters were interesterified in HCl-methanol and the methyl esters submitted according to the procedure of Stoffel et al. (12). Gas-liquid chromatography was carried out as previously described (6) with a succinate polyester of diethylene glycol as the stationary phase (13).

RESULTS

Normal Fasted Rats—The fatty acid composition of the cholesterol esters of normal rat serum and liver are shown in Table I. In the serum, the polyunsaturated fatty acids comprised 68.8% of the total cholesterol ester fatty acids. The major acid in this group was arachidonic acid which made up 50% of the total acids. The short chain (C\textsubscript{4} to C\textsubscript{12}) and saturated fatty acids (myristic, palmitic, and stearic) accounted for 17.5% of the total with the major acid in this group being palmitic. The monoenes made up only a small fraction of the total fatty acids (12.7%). The composition of the cholesterol ester fatty acid fraction in the liver (Table I) was quite different from that of the serum. In the former, the polyunsaturated fatty acids made up only 31.2% of the total. The short chain and saturated fatty acids (myristic, palmitic, and stearic) comprised 55.5% of the total fatty acids; the major fatty acid of the liver esters was palmitic acid (32.5%).

For another type of comparison of the cholesterol ester fatty acid composition of serum and liver, the amounts of the cholesterol ester fatty acids in the serum and liver were computed; the average results are shown in Table II. The serum had approximately twice (3.06 mg) as much cholesterol ester fatty acids as the liver (1.52 mg). In respect to the individual fatty acids, the...
**Table I**

Cholesterol ester fatty acid composition of serum and liver in normal and lymph-fistula rats

<table>
<thead>
<tr>
<th>Fatty acid*</th>
<th>Normal†</th>
<th>Lympf-fistula†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chain length</td>
<td>No. of double bonds</td>
<td>Serum</td>
</tr>
<tr>
<td>6 to 12</td>
<td>1.7 ± 0.4</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>14</td>
<td>0.8 ± 0.2</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>16</td>
<td>0.3 ± 0.3</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>18</td>
<td>12.4 ± 2.1</td>
<td>22.5 ± 2.5</td>
</tr>
<tr>
<td>18</td>
<td>1.2 ± 1.1</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td>18</td>
<td>0.6 ± 0.3</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>18</td>
<td>1.6 ± 0.0</td>
<td>20.1 ± 4.4</td>
</tr>
<tr>
<td>20</td>
<td>50.0 ± 0.9</td>
<td>8.8 ± 1.9</td>
</tr>
</tbody>
</table>

* Represents the major fatty acids found. Very small amounts of 16:2, 20:0, 20:3 and 20:5 and others were also observed.
† Values represent the average of 5 to 6 rats plus or minus the standard deviation.

**Table II**

Cholesterol ester fatty acid contents of serum and liver in normal and lymph-fistula rats

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Normal*</th>
<th>Lympf-fistula*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chain length</td>
<td>No. double bonds</td>
<td>Serum</td>
</tr>
<tr>
<td>6 to 12</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>14</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>16</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>16</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>18</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>19</td>
<td>0.80</td>
<td>0.19</td>
</tr>
<tr>
<td>18</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20</td>
<td>1.50</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* The mg of the individual cholesterol ester fatty acids in the liver were calculated from the total cholesterol ester fatty acid content of the liver and the percentage of the individual acids as indicated in Table I. For serum the amount of individual acids in the ester fraction per ml was calculated as for liver and then multiplied by the serum volume.

The present study has provided more definitive information regarding the distribution of the fatty acids in the cholesterol ester fraction of plasma and liver in fasted rats than has been previously available. The data indicate the presence of approximately 10% oleic acid in the serum cholesterol esters of fasted rats, which is in agreement with the report of Mukherjee et al. (1) that oleic acid is absent from this fraction, or with the report of Klein and Janssen (2) that oleic acid comprises 59% of the acids. These discordant results may be due to the use of fasted rather than fed animals or to differences in the analytical techniques.

The results of the present study also show that in normal rats the liver and serum cholesterol esters differ widely in their composition. The differences in the fatty acid composition between the two cholesterol ester pools suggest the possibility that there may be a selective exchange of the different cholesterol esters between the liver and the blood. In accord with this, recent data by Klein and Martin (14) suggest that the different liver cholesterol esters have different turnover rates. However, the comparative data on the normal and lymph-fistula animals are also in agreement with an alternative possibility, namely, that in the normal animal the serum cholesterol ester level and composition is controlled in part by the cholesterol ester coming by way of the lymph. When lymph is diverted, there is an increased exchange of unsaturated fatty acid esters (linoleic, arachidonic, and oleic acids) between the liver and the blood. The data of an earlier study (7) indicated that in the normal rat a large portion of the blood cholesterol esters originated in the intestine. When lymph cholesterol was diverted from the blood, by way of a fistula, the synthesis of both free and esterified cholesterol from C incorporation was greatly increased. However, in the lymph-fistula rats the cholesterol ester content of the blood was maintained at its normal level, whereas that fraction of the liver decreased as in the present experiments. Confirmatory evidence was ob-

**DISCUSSION**

The greatest differences were in the polyunsaturated group; approximately 12 times more arachidonic and 3 times more linoleic acid were present in the serum than in the liver. Palmitic and oleic acids were present in approximately equal amounts, but the amount of stearic in the liver ester fraction was 4 times greater than in the serum.

**Normal versus Lymph-fistula Rate**—Comparable data on the serum and liver cholesterol ester fatty acid composition of lymph-fistula rats are also shown in Tables I and II. In the lymph-fistula group also, a major portion of the serum cholesterol fatty acids was present as polyunsaturated acids (51.5%), but there was a lower percentage of arachidonic acid in comparison to the normal animals. This drop in the percentage of arachidonic acid of the cholesterol ester fatty acids in the lymph-fistula animal was approximately equal to the sum of the increases in saturated acids and oleic acid. That this represented an actual replacement of arachidonic acid in the serum cholesterol ester fatty acids by saturated and monoenoic acids is shown in Table II. There was virtually no change in the total amount of serum cholesterol ester fatty acids in the lymph-fistula animal as compared to the normal animal. However, there was a considerable decrease in the amount of arachidonic acid and an increase in the saturated acids and oleic acid. When the amounts of liver cholesterol ester fatty acids are compared in the two types of animals (Table II), it can be seen that the levels of palmitic and stearic acids were the same, but there was a decline in the amounts of oleic, linoleic, and arachidonic acids in the lymph-fistula animals.

Of the latter acids, oleic acid showed the greatest decrease (67.8%). Thus, in contrast to the serum cholesterol ester fatty acid level, which was approximately the same in both types of animals, the liver fraction of the lymph-fistula animal showed a 28.9% drop.
tained in a more recent study in man (8) in which it was shown that the turnover rate of liver cholesterol esters is insufficient to account for the observed turnover rate of this fraction in the plasma.

Of particular interest is the observation that normal fasted rats - serum cholesterol ester fatty acids have a very high content of arachidonic acid (50%). Other studies (1, 2), with the aid of the alkali isomerization technique, have also reported high levels of polyunsaturated fatty acids in rat plasma cholesterol esters. In contrast to these findings in the rat, the major fatty acid of the cholesterol ester fraction of human serum is linoleic acid, with arachidonic acid constituting only 5 to 10% of the total fatty acids (2, 6, 16). It has been hypothesized (17) that a deficiency of the factors needed for the synthesis of arachidonic acid, and linoleic acid, and pyridoxine, may be an important factor in the development of atherosclerosis in man. The very large proportion of arachidonic acid in the serum cholesterol ester fatty acids of the rat and the resistance of this animal (18, 19) to experimentally produced atherosclerosis suggest the importance of further examination of that hypothesis. Arachidonic acid may have a significant role in transporting cholesterol in and out of the tissues. The rabbit, which is highly susceptible to the production of atherosclerosis by dietary means, has a very low content of arachidonic acid in the serum cholesterol esters. Another factor contributing to the difference in arachidonic acid levels between the rat and man may be the different dietary habits of the two species since man generally consumes diets containing approximately 30% fat, whereas the usual rat pellet chow contains only 5% fat.

SUMMARY

1. The cholesterol ester fatty acid composition of the serum and liver of normal and lymph-fistula rats were determined by gas-liquid chromatography.
2. The serum and liver of the normal animal are distinctively different in their cholesterol ester fatty acid composition. Up to 70% of the total cholesterol ester fatty acids in the serum were polyunsaturated. The major acid of normal rat serum cholesterol esters was arachidonic acid (50%). The major fatty acids of the liver were of the saturated and monoenoic type with palmitic acid predominating (32.5%).
3. Removal of lymph cholesterol by way of a fistula decreased the amount of arachidonic acid in the serum with a proportionate increase in the percentage of saturated and oleic acids. The liver of those animals showed a drop in the total cholesterol ester content and in the percentage of polyunsaturated fatty acids in the ester fraction.
4. The results of the present study suggest that both the liver and the lymph cholesterol ester play an important role in the regulation of the blood cholesterol ester level and composition.

REFERENCES

Cholesterol Ester Fatty Acids in Serum and Liver of Normal and Lymph-fistula Rats
Leon Swell, M. D. Law, Henry Field, Jr. and C. R. Treadwell