Fatty Acid Composition of Lysophosphatidylcholine, Phosphatidylinositol and Phosphatidylserine of Human Lipoprotein (a)- A Comparison with Low Density Lipoprotein

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Edited by T. Yanagita, Saga Univ., and accepted June 26, 2003 (received for review May 1, 2003)

Abstract: The fatty acid composition of lipoprotein (a) is known for the cholesteryl ester, triglyceride and the total and major individual phospholipid fractions. Minor phospholipids’ fatty acids are unknown for lipoprotein (a). The minor phospholipids include phosphatidylinositol, phosphatidylserine and lysophosphatidylcholine. This study sought to elucidate the fatty acid composition of the minor individual phospholipids of lipoprotein (a). For validation and comparison, low density lipoprotein was from lipoprotein (a) donors’ blood. Palmitic acid was the major fatty acid in all minor phospholipids except phosphatidylinositol where stearic acid was the largest contributor for Lp(a).

Key words: Lp(a), LDL, minor phospholipids’ fatty acid composition

1 Introduction

Lipoproteins have their lipids in two categories - neutral and the phospholipids. The main neutral lipids are cholesteryl esters (CE) and triglycerides (TG). The total phospholipids (PL) consist of choline-containing phospholipids (PC), lyso-phosphatidylcholine (LPC), ethanolamine-containing phospholipids (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SPH). The physiological significance of Lp(a) is undetermined. However, lipoprotein (a) (Lp(a)) and low density lipoprotein (LDL) are pro-atherogenic (1,2). The fatty acid composition of LDL has been related to their atherogenic role. For example the linoleic acid (LA, 18:2 n-6) content of LDL has been related to plasma cholesterol (3). Further oxidized LDL is considered to be highly atherogenic (4). The fatty acid composition of a lipoprotein in part dictates its ability to undergo oxidation (5). The mono- and poly-unsaturated fatty acid content relative to the saturated fatty acid content can clearly impact degree of oxidation. LPC has been given an atherogenic role (6), though the impact of its fatty acid composition in that regard is not clear from the literature.

Published reports on the fatty acid composition of Lp(a) have elucidated the fatty acids of cholesteryl ester, triglycerides, triglycerides and the total phospholipids including the major individual phospholipids (7,8). The minor individual phospholipids (LPC, PS and PI) of Lp(a) have never been assessed with regard to fatty acid composition.

Thus, it was the objective of this study to elucidate for the first time the fatty acid composition of the minor individual phospholipids of Lp(a). It was also of interest to examine the data within the various lipid groups of Lp(a) to determine any differences compared to LDL. LDL with more thoroughly established fatty acid compositions and a shared pro-atherogenic ability with Lp(a) was chosen for the purposes of quality control and comparison.

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It was hypothesized that there should be only modest or no differences in fatty acid composition for a given lipid fraction between the two lipoproteins given that fatty acid composition is based on dietary fatty acids, metabolic regulation of fatty acids and preferential incorporation of fatty acids (9). Diet can be ruled out since LDL and Lp(a) were from the same patients and the latter two should be reasonably similar at least from the perspective that Lp(a) and LDL are assembled and secreted by the liver (10). Of course, lipoprotein metabolism is much more complex than assembly and secretion.

2 Materials and Methods

The subjects were 6 healthy male volunteers with a mean age of 42 years. Their diet was determined by a seven day dietary record and found to contain 2395 kcal per day made up of 50, 32 and 18 percent carbohydrate, fat and protein respectively. The main fatty acids in the diet were 14:0 (1.5), 16:0 (6.2) 18:0 (2.4), 18:1 (12.9), 18:2 (6.3)(percent of kilocalories). The Human Ethics Committee of the University of Pennsylvania gave certification for the study and the subjects signed an informed consent. Analysis was done at the Renal Division of Baxter Healthcare Corporation.

Lipid standards (LPC, PS, and PI) for thin layer chromatography were obtained from NuChek Prep (Elysian, MN). Silica gel 60 pre-coated glass plates (0.25 mm. thickness) were purchased from Merck (Gibbstown, N.J.). All other chemicals and solvents were ACS certified and obtained from Fisher (Dallas, TX).

Approximately 100 mL of blood were drawn from the antecubital veins. Plasma was prepared by centrifuging the blood at 1000 × g for 15 min at 30°C. LDL and Lp(a) were isolated from the same subjects according the methods of Kostner et al. (11) and Seman et al. (12) respectively. Briefly, LDL was separated by ultracentrifugation and Lp(a) was isolated by lectin chromatography. This lectin chromatography approach is Lp(a) isoform independent (12). Purity of each of the lipoproteins was assured via rocket electric electrophoresis and SDS-PAGE (Gaubatz et al. 1983 (13), Laurell 1966 (14) and Armstrong et al. 1985 (15)).

Lipoproteins were extracted according to the method of Folch et al. (16) using 400 μl of concentrate containing purified lipoprotein followed by 8 ml of chloroform/methanol (2:1 v/v) followed by 1.4 ml of 0.88 % KCl. After evaporation under N₂, lipid extracts were applied to silica gel 60 pre-coated glass plates and the LPC, PS, and PI were isolated by thin layer chromatography (TLC)(17) followed by boron trifluoride: methanol transmethylation and subsequent GLC analysis of the purified FAMES (7,17). Prior to GLC, the FAMES were purified using toluene as the developing solvent on silica gel 60 plates and eluted using petroleum ether. Gas liquid chromatography was done using a Hewlett Packard (HP) 5890A gas liquid chromatographic instrument with a HP 3393A integrator. The flow rate was 1.5 ml/min using a Supelco Omegawax 250 fused silica capillary column of 30 m in length, 0.25 mm internal diameter, and a 0.25 mm film thickness. The runs were 50 minutes at an oven temperature of 210°C using helium as the carrier gas and compressed air and hydrogen for the flame ionization detector (FID). The FID was set at 260°C. Rapid extraction of fresh Lp(a) and LDL from fresh plasma followed by immediate TLC, methylation, and GLC minimized the opportunity for fatty acid oxidation.

The data were assessed with a student’s T-test using single pairs option. The level of significance was selected at P < 0.05.

3 Results

The fatty acid composition of lyso-PC is shown in Table 1. The major (> 5 mol%) fatty acids were PA, SA, and OA. LDL was considerably more enriched in SA than OA with the opposite being true for Lp(a).

The fatty acid composition of PS is shown in Table 2. The major fatty acids were PA, SA, LA, and OA.

The fatty acid composition of PI is shown in Table 3. The major fatty acids were SA, AA, PA, ALA, AA and LA. Among these, LDL was more enriched than Lp(a) in all but ALA.

4 Discussion

The fatty acid composition of LDL showed consistency with the literature (18-25) thus validating the LDL work, giving credence to the Lp(a) fatty acid compositions, and suggesting non-existent or minimal fatty acid oxidation. Further validity is offered by the presence of all fatty acids found in the individual phospholipids occurring in the total phospholipid fraction.
The fatty acid composition of Lp(a)’s minor phospholipids

The overall fatty acid composition of total Lp(a) and LDL have been reported to be statistically identical (7). While the current work indicates that there are many similarities in the fatty acid compositions of LDL and Lp(a), it cannot be said that the similarities are as reported by Sattler et al. (7) extend to the minor individual phospholipids. It may be that a larger pool or different group of subjects or the revelation of more fatty acids resulted in the differences between this paper and that of the Sattler group (7).

This is the first paper to examine the fatty acid compositions of the minor individual phospholipids of Lp(a). It is also a first examination of the comparison of individual phospholipids between LDL and Lp(a).

Regarding the overall data for LDL, LPC (Table 1) is consistent with total serum LPC (26). Lyso-PC has long been of interest because of its purported roles in atherosclerotic risk. Various mechanisms have been proposed (6, 27) though the impact of the fatty acid composition of LPC has not been made clear. The sharp contrasts in the fatty acid composition of LPC between LDL and LP(a) and the subsequent contributions of these differences to the putative roles of LPC in atherogenicity remain an area to be investigated.

PS (Table 2) has a far lower relative amount of AA in Lp(a) than LDL. Unlike PE, PS has no sharp differences in the amount of SA, OA, and LA between Lp(a)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Lp(a)</th>
<th>LDL</th>
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<tbody>
<tr>
<td>14:0</td>
<td>6.0 ± 1.5</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>14:1</td>
<td>3.8 ± 0.3a</td>
<td>0.8 ± 0.1b</td>
</tr>
<tr>
<td>16:0</td>
<td>29.2 ± 3.2</td>
<td>33.3 ± 4.3</td>
</tr>
<tr>
<td>16:1</td>
<td>4.4 ± 0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>27.2 ± 2.8</td>
<td>27.1 ± 3.2</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>7.6 ± 1.9</td>
<td>5.9 ± 0.4</td>
</tr>
<tr>
<td>18:2</td>
<td>16.9 ± 1.8</td>
<td>12.9 ± 4.2</td>
</tr>
<tr>
<td>18:3 n-6</td>
<td>2.7 ± 1.3</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>0.6 ± 0.1a</td>
<td>0.1 ± 0.02b</td>
</tr>
<tr>
<td>20:0</td>
<td>0.0</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>20:3 n-6</td>
<td>0.6 ± 0.1a</td>
<td>1.7 ± 0.3b</td>
</tr>
<tr>
<td>20:4</td>
<td>0.8 ± 0.1a</td>
<td>9.3 ± 0.9b</td>
</tr>
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All values represent the means ± SEM for 6 subjects. Comparisons are for Lp(a) vs LDL for a given fatty acid. Values across a given row having different superscript letters are significantly different (p < 0.05).

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<td>6.0 ± 0.7a</td>
<td>1.6 ± 0.2b</td>
</tr>
<tr>
<td>14:1</td>
<td>2.4 ± 0.2a</td>
<td>0.4 ± 0.1b</td>
</tr>
<tr>
<td>16:0</td>
<td>43.5 ± 8.7</td>
<td>49.1 ± 1.4</td>
</tr>
<tr>
<td>16:1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>10.8 ± 1.7a</td>
<td>33.6 ± 7.9b</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td>31.8 ± 3.0a</td>
<td>12.6 ± 2.4b</td>
</tr>
<tr>
<td>18:2</td>
<td>1.1 ± 0.2a</td>
<td>0.6 ± 0.1b</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>1.7 ± 0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>20:4</td>
<td>0.0</td>
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and LDL.

PL (Table 3) in LDL is like PE in terms of having higher levels of LA and AA compared to Lp(a). PL is also like most of the PL fractions for LDL compared to Lp(a) in having a tendency to accumulate PUFA which may explain the greater tendency of LDL toward oxidation (7).

The differences between the individual PL fractions are almost consistently reflected in higher accumulations of PUFA for LDL or Lp(a) which may also relate to the greater LDL fluidity (28).

Differences in fatty acid compositions of various lipid fractions depend upon their ability to preferentially incorporate certain fatty acids, dietary fatty acids, and metabolic regulation of fatty acid synthesis (8). As Lp(a) and LDL were from the same subjects and both lipoproteins are assembled in the liver, similar metabolic regulation and an ability to incorporate fatty acids into a given fraction is strongly suggested (9). Indeed, most differences were minor (less than 5 mol %). The similarities could also represent exchange equilibrium between the two lipoproteins (20) with the very few major differences (greater than 5 mol %) representing only 10% of all elucidated fatty acids and perhaps indicating a molecular species disequilibrium among identical lipid fractions between the two lipoproteins. The differences in fatty acid composition between different phospholipids in each of the lipoproteins may depend upon their ability to preferentially incorporate certain fatty acids, dietary fatty acids, and the metabolic regulation of fatty acid synthesis (9).

In conclusion, while there are many similarities in the fatty acid composition between Lp(a) and LDL, as predicted by the hypothesis there are a few sharp differences both between Lp(a) and LDL. The TG and CE fractions differed slightly when Lp(a) was compared to LDL with differences being more pronounced in the PL fractions. LDL appears to have a preference for the n-3 and n-6 PUFA in total and most of the individual PL fractions. LDL also has a preference for the PL accumulation of the longer chain saturated and monounsaturated fatty acids except for PC. The significance of the fatty acid composition in each of the lipid fractions remains to be fully elucidated. As the true physiological functions of Lp(a) and its’ atherogenicity relative to LDL to are currently unknown, it is impossible to know the significance of the impact of differences between the two lipoproteins in fatty acid composition for a given lipid fraction. It would be of great interest to determine if these preferential accumulations of the various fatty acids in some of the lipid fractions of the two lipoproteins impacts their relative atherogenicity via such factors as oxidation potential, impact upon cellular signaling (29-34) and lipoprotein fluidity.

References