Composition and electrophoretic mobility of plasma lipoproteins of dromedary camels (Camelus dromedarius)

Farzad Asadi, DVM, PhD; Ali Shahriri, DVM, PhD; Peyman Asadian, DVM, PhD; Maltie Pourkabir, DVM, PhD; Mahzyar Samadpour, DVM

Objective—To determine the lipid composition and electrophoretic pattern of plasma lipoproteins in samples obtained from healthy 1-humped camels (Camelus dromedarius).

Animals—34 healthy camels raised under similar farming and dietary conditions.

Procedures—Plasma samples were subjected to density-gradient ultracentrifugation for separation of plasma lipoproteins, including very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Purity of the separation was assessed by use of polyacrylamide gel disk electrophoresis. Concentrations of triglycerides, cholesterol, and phospholipids were measured in each lipoprotein fraction, and lipoprotein electrophoretic patterns were determined in plasma samples.

Results—Phospholipid was the major constituent of LDL [(mean ± SD concentration, 10.62 ± 1.2 mg/dL), LDL (24.66 ± 3.12 mg/dL), and HDL (38.08 ± 0.76 mg/dL). Low-density lipoprotein, VLDL, and HDL were important plasma lipoprotein carriers for cholesterol (67.94 ± 9.51%), triglyceride (55.63 ± 78.1%), and phospholipid (51.91 ± 15.5%), respectively. On the basis of electrophoretic results, relative percentages of α- and β-lipoproteins were 31.72 ± 4.88% and 65.3 ± 4.88%, respectively.

Conclusions and Clinical Relevance—The lipoprotein profile in 1-humped camels differed substantially from that of other ruminants. Results may be useful in the evaluation and treatment of metabolic disorders in camels. (Am J Vet Res 2008;69:880-885)

The medical literature contains ample evidence that abnormal lipoprotein metabolism is strongly associated with development of atherosclerotic vascular disease.12 Accordingly, lipoprotein analysis has become an essential part of routine laboratory investigation. Lipoprotein profiles have been investigated in numerous animal species.16 Results of one of those studies7 reveal that important variations exist among species and even within families. It has been proposed that investigations involving several animals can yield valuable information about lipid metabolism in the species being examined and also contribute to a better understanding of the pathophysiologic aspects of hypercholesterolemia and atherosclerosis.

In domestic animals, certain plasma lipoprotein fractions, namely the VLDLs (i.e., pre-β-lipoprotein), LDLs (i.e., β-lipoprotein), and HDLs (i.e., α-lipoprotein), may share similarities with those found in humans. In animals such as guinea pigs, which have been described as LDL animals, analysis of the lipidogram could reveal a lack of HDL, whereas in cattle and goats, which have been described as HDL animals, analysis of the lipidogram reveals a total lack or only trace quantities of LDL.8

In most studies in which animals have been used experimentally, it has been assumed that the density of lipoproteins was identical to the lipoprotein density in humans. However, there is evidence that the density of serum lipoproteins in animals differs from that in humans.9 By means of density-gradient ultracentrifugation and premitting of the serum lipoproteins, it has been revealed that animal species vary widely in various aspects of the lipoprotein profile.8,9

Broadly speaking, there are 2 species of camel (the Arabian camel, also known as the 1-humped or dromedary camel [Camelus dromedarius], and the Bactrian camel, also known as the 2-humped camel [Camelus bactrianus]). Dromedary camels inhabit southwestern Asia and northern Africa and have been introduced into parts

<table>
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<th>ABBREVIATIONS</th>
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<tr>
<td>HDL</td>
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<td>LDL</td>
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<td>VLDL</td>
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of North America and Australia. These ruminants are accustomed to difficult environments, such as rugged mountains and desert terrain. In Iran, camels primarily live in the eastern and northeastern desert regions. In a 1971 study, serum lipoproteins of Bactrian camels were investigated. Subsequently, 3 other reports on serum lipid concentrations in camels were published. In those studies, the cholesterol and triglyceride concentrations in lipoproteins were measured with commercially available enzymatic kits. In one of those studies, plasma HDL fractions were harvested (as supernatant) by precipitating the apoprotein-containing lipoproteins. Similarly, use of the Friedewald formula revealed that the VLDL-cholesterol fraction was estimated as being a fifth of the triglyceride concentration. The LDL-cholesterol fraction was calculated as the approximate difference between the cholesterol content in the precipitate and that in the HDL fraction. To obtain more accurate values of these lipid variables, we believe that a more refined technique for isolating lipoproteins (ie, ultracentrifugation) is necessary.

Despite increases in knowledge regarding lipid metabolism and transport in various animal species, the plasma lipoprotein distribution and composition in dromedary camels have not yet been fully examined. The objective of the study reported here was to investigate the lipid profile and electrophoretic mobility of lipoproteins in plasma samples obtained from dromedary camels.

Materials and Methods

Thirty-four client-owned dromedary camels raised under similar conditions were used in the study. Owner consent was obtained for inclusion of each camel used in the study. Camels resided in the Sistan and Balochestan desert areas at the eastern borders of Iran and were fed dry hay daily. Camels had ad libitum access to water, but fodder was withheld for approximately 14 hours before blood collection.

Blood samples were collected in mid-August 2006. Camels were manually restrained without sedation, and 10 mL of blood was collected into disodium EDTA-containing evacuated tubes via an incision made on the neck over a jugular vein. Plasma was immediately separated by centrifugation of samples in a tabletop centrifuge at 1,000 X g for 15 minutes. Plasma was then harvested, and samples were kept on ice throughout the manipulation and stored at -80°C until analysis. All samples were analyzed within 1 month after plasma was obtained.

Separation of plasma lipoproteins was performed in accordance with a method described elsewhere, with a few modifications. Briefly, a density gradient was formed by adjusting 2 mL of plasma to a density of 1.210 g/mL by the addition of 1.09 g of KBr, 0.71 g of sucrose, and 0.2 mL of freshly prepared Sudan black solution (1 g/L in polyethylene glycol). The gradient was sequentially layered with 2 mL of NaCl-KBr solutions with a density of 1.063 g/mL, 4 mL of NaCl-KBr solution with a density of 1.019 g/mL, and 4 mL of distilled water with a density of 1.000 g/mL. All solutions contained 1 X 10^{-4} g of sodium EDTA/mL. Density of the generated fractions was determined by use of an analytic balance. Density of each stock solution was determined by use of a densitometer. Samples in tubes were centrifuged in a swinging bucket rotor at 270,000 X g for 22 hours at 20°C. After centrifugation, lipoproteins were separated, and corresponding bands were aspirated with a rubber-bulb pipette in accordance with a technique described elsewhere. Lipoprotein fractions were dialyzed overnight against 0.15M NaCl and 1mM EDTA (pH 7.0) at 4°C by use of dialysis tubing. To assess purity of the separation, a 20-μL aliquot of each fraction was evaluated via polyacrylamide gel disk electrophoresis (3 mA/tube for approx 40 minutes) in accordance with a method described elsewhere. Lipoprotein bands were immediately recorded by a scanner.

For compositional analysis, lipids in all lipoprotein fractions were extracted separately. The concentration of cholesterol in the lipoproteins was also measured in accordance with a method described elsewhere. Calibrated serum (4 mg/dL) was used for cholesterol standards. Triglyceride concentrations were determined by means of triglyceride mass assay. Phospholipid concentrations were measured in accordance with a method described in another report. Electrophoresis of plasma lipoproteins was performed in accordance with a method described elsewhere, with slight modifications. Briefly, agar-agarose gel was prepared by mixing 0.5% agarose, 0.6% agar, and bovine serum albumin (2.5 g/L) at a ratio of 4:1.0:1 (vol:vol:vol). Electrophoresis was conducted at 1.20 V at 10°C for 2.5 hours. Gels were fixed in 30% methanol solution and dried overnight at 60°C. Band densities were recorded by staining with Sudan black stain (0.4 g of Sudan black, 120 mL of ethanol solution, + g of zinc acetate, and 80 mL of distilled water) for 2 hours, which was followed by destaining with tap water. All gels were scanned at 570 nm by use of a lipoprotein scan densitometer.

Results

The density profile of serum lipoproteins in dromedary camels after separation by density-gradient ultracentrifugation was recorded (Figure 1). Two heavily stained lipoprotein bands were detected (the first in the density range of 1.006 to 1.063 g/mL and the second in the density range of 1.063 to 1.210 g/mL). A minor band in the lower-density range (density < 1.006 g/mL) was also detected. Bands for each lipoprotein fraction isolated by use of polyacrylamide gel disk electrophoresis were detected (Figure 2).

Results of lipid analysis of each fraction were summarized (Table 1). The proportion of various lipids in each fraction was tabulated (Table 2). Each value represented the arithmetic mean ± SD for the 34 values expressed as a percentage, which was obtained by calculating the total amount of the lipid class recovered in the 3 lipoprotein fractions and determining the percentage of that lipid class found in each fraction. For example, the lipoprotein distribution of triglyceride was calculated as follows: (triglyceride/[VLDL - triglyceride] + [LDL - triglyceride] + [HDL - triglyceride]) X 100. Most of the triglyceride and phospholipid was found in the VLDL and HDL fractions, respectively.
Figure 1—Density profile of plasma lipoproteins after density-gradient ultracentrifugation in a sample obtained from a dromedary (1-humped) camel (Camelus dromedarius).

Figure 2—Lipoprotein bands (arrows) of each fraction harvested from the density-gradient ultracentrifugation. Notice the origin for each sample before ultracentrifugation and the resulting bands (HDL, LDL, VLDL, and HDL, right).

Table 1—Mean ± SD values for plasma lipoprotein composition in samples obtained from 34 dromedary (1-humped) camels (Camelus dromedarius).

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>Phospholipid (mg/dL)</th>
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<tbody>
<tr>
<td>VLDL</td>
<td>0.60 ± 0.10</td>
<td>7.33 ± 0.80</td>
<td>10.62 ± 1.20</td>
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<tr>
<td>LDL</td>
<td>6.23 ± 0.68</td>
<td>3.24 ± 0.49</td>
<td>24.66 ± 3.12</td>
</tr>
<tr>
<td>HDL</td>
<td>2.04 ± 0.38</td>
<td>2.56 ± 0.46</td>
<td>38.05 ± 0.76</td>
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*Lipoprotein fractions were separated via density-gradient ultracentrifugation. Density for each of the lipoprotein fractions was as follows: VLDL < 1.006 g/mL, LDL 1.006 to 1.056 g/mL, and HDL, 1.063 to 1.210 g/mL.

Table 2—Mean ± SD values for distribution of lipid class in lipoprotein fractions of plasma samples obtained from 34 dromedary camels.

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Cholesterol (%)</th>
<th>Triglyceride (%)</th>
<th>Phospholipid (%)</th>
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<tbody>
<tr>
<td>VLDL</td>
<td>16.81 ± 2.85</td>
<td>74.07 ± 13.33</td>
<td>8.57 ± 2.65</td>
</tr>
<tr>
<td>LDL</td>
<td>58.59 ± 7.60</td>
<td>16.59 ± 2.25</td>
<td>22.70 ± 2.69</td>
</tr>
<tr>
<td>HDL</td>
<td>24.70 ± 4.69</td>
<td>10.44 ± 2.68</td>
<td>37.11 ± 0.37</td>
</tr>
</tbody>
</table>

Each value represents the arithmetic mean ± SD for the 34 samples expressed as a percentage. Values were obtained by calculating the total amount of the lipid class recovered in the 3 lipoprotein fractions and determining the percentage of that lipid class found in each fraction. For example, the lipid class distribution of triglyceride was calculated as follows: triglyceride/(VLDL - triglyceride) + (LDL - triglyceride) + (HDL - triglyceride)) X 100.

See Table 1 for remainder of key.
Table 3—Mean ± SD values for composition of lipid class in lipoprotein fractions of plasma samples obtained from 34 dromedary camels.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
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<tbody>
<tr>
<td>Cholesterol (%)</td>
<td>9.81 ± 0.78</td>
<td>67.94 ± 9.51</td>
<td>22.25 ± 1.55</td>
</tr>
<tr>
<td>Triglyceride (%)</td>
<td>55.83 ± 7.81</td>
<td>24.65 ± 1.97</td>
<td>19.59 ± 1.37</td>
</tr>
<tr>
<td>Phospholipid (%)</td>
<td>13.48 ± 1.16</td>
<td>33.62 ± 2.35</td>
<td>51.91 ± 1.55</td>
</tr>
</tbody>
</table>

Each value represents the arithmetic mean ± SD for the 34 samples expressed as a percentage. Values were obtained by calculating the total lipid in each lipoprotein fraction and determining the percentage contributed by each lipid class in that fraction. For example, the lipid class composition of triglyceride was calculated as follows: (triglyceride/[VLDL - cholesterol] + [VLDL - triglyceride] + [VLDL - phospholipid]) × 100.

See Table 1 for remainder of key.

Figure 3—Plasma lipoprotein patterns after electrophoresis for samples obtained from dromedary camels. For the VLDL (left), LDL (center), and HDL (right) bands, densitometric peaks indicate the α-lipoprotein and β-lipoprotein regions. -- = Anode; + = Cathode.

The proportion of each lipid distribution in the lipoprotein fractions was determined (Table 3). Each value represented the arithmetic mean ± SD for the 34 samples expressed as a percentage. Values were obtained by calculating the total lipid in each lipoprotein fraction and determining the percentage contributed by each lipid class in that fraction. For example, the lipid class composition of triglyceride was calculated as follows: (triglyceride/[VLDL - cholesterol] + [VLDL - triglyceride] + [VLDL - phospholipid]) × 100.

Electrophoretic mobility of extracted plasma lipoproteins in dromedary camels was determined (Figure 3, Table 3). Two important electrophoretic regions were evident (a β-lipoprotein region and an α-lipoprotein region, with the former comprising a larger area). Mean ± SD relative percentages of α- and β-lipoproteins were 31.72 ± 4.88% and 68.30 ± 4.66%, respectively; thus, the mean ratio of β-lipoprotein to α-lipoprotein was 2.23 ± 0.54.

Discussion

To the authors’ knowledge, the study reported here was the first in which density-gradient ultracentrifugation was used to characterize lipoproteins in camel plasma. The 2 heavily stained bands in the density range of 1.063 to 1.210 g/mL and 1.006 to 1.063 g/mL represented the HDL and LDL fractions, respectively. Similar density ranges for these lipoproteins have been reported in guinea pigs, rabbits, and rats. All of those animals have a faint band in the VLDL density range. The identity of the band at a density of 1.006 g/mL (i.e., top of the gradient) was unknown.

The percentage of cholesterol in the HDL fraction of dromedary camels (approx 22%) is substantially less than that reported in rats (50%), goats (45%), swine (36%), cattle (73%), horses (60%), deer (52%), mink (60%), and foxes (65%). However, it appears to be comparable to the percentage reported in guinea pigs (29.3%), rabbits (27%), sheep (24%), and wild boars (30%).

Similarly, the percentage of phospholipid in the HDL fraction of dromedary camels (approx 52%) differs from the value reported in guinea pigs (42%), rabbits (63%), rats (57%), sheep (63%), goats (64%), swine (60%), cattle (77%), horses (83.7%), deer (76%), wild boars (71%), mink (61%), and foxes (68%).

Interestingly, the percentage of cholesterol in the LDL fraction of camels (approx 68%) is comparable to that in humans (70% to 75%), guinea pigs (70%), rabbits (73%), sheep (76%), swine (64%), and wild boars (70%). However, the value in camels is much higher than that reported in rats (50%), goats (55%), cattle (27%), horses (40%), deer (48%), mink (40%), and foxes (35%).

The percentage of phospholipid in the LDL fraction (approx 34%) is also similar to values reported in humans (40%), rabbits (37%), sheep (35%), goats (36%), swine (40%), and foxes (32%). Although the percentage is higher than that in cattle (23%), horses (17%), and deer (24%), it is lower than that in guinea pigs (38%) and rats (43%).

Finally, the percentage of cholesterol in the VLDL fraction of dromedary camels (approx 10%) is similar to that in rhesus monkeys (13% to 16%), humans (5.5% to 8.5%), and guinea pigs (10%). However, the percentage in dromedary camels is lower than the percentage in rabbits (27%) but higher than the percentage in pigs (1.5%), horses (1.4% to 3.3%), sheep (1%), goats (4.5%), cows (1% to 1.5%), and rats (2%).
The lipoprotein lipid profile of humans has been amply described. In brief, the mean percentages of triglyceride, cholesterol, and phospholipid in VLDL (50%, 29%, and 20%, respectively), LDL (13%, 60%, and 27%, respectively), and HDL (12%, 45%, and 43%, respectively) fractions differ substantially from values detected in dromedary camels.

It has been suggested that variations in activities of plasma cholesteryl ester transferase and phospholipid transferase on lipoprotein substrates may explain the diversity in lipid composition of lipoproteins among vertebrate species. However, it is possible that the differences are an erroneous result of differences in experimental procedures. For instance, there is disagreement among investigators regarding the effects of freezing and thawing on the lipoprotein profile. In a study, freezing serum at -70°C for 1 month did not affect the quantity of HDL cholesterol. In another study, investigators determined that LDL does not remain intact after a single freeze-thaw cycle, and evidence has been provided that values for lipoproteins remain consistent for 18 months when serum is stored at a constant temperature of -20°C. However, in that latter study, lipid concentrations progressively decreased with large fluctuations in storage temperature. Similarly, storage of plasma for up to 10 days with a single freeze-thaw cycle does not affect the reproducibility of lipoprotein cholesterol analysis. In contrast, it was proposed in another study that the HDL structure is not stable during freezing and thawing. Stability of lipoproteins and their optimal storage conditions remain to be elucidated.

Evaluation of the lipogram of camel plasma separated on agarase-gel agar revealed that the primary constituents were β-lipoproteins. The proportion of the β-lipoprotein region in camels was similar to the proportion reported in rats (61%), domestic swine (69%); wild bears (68%); and goats, cattle, and mink (70%). It has been reported that electrophoresis of serum lipoproteins conducted with different supporting media, such as agarose and cellulose acetate, could yield differences in mobility patterns and detection of 1 or more additional bands in the pre-B- or post-α-regions. Although separation of lipoproteins via SDS-PAGE is influenced mainly on the basis of size, the authors have adopted the agarose-agarose system in which macromolecules are separated on the basis of both ionic charge and size.

A discrepancy was detected between results of the study reported here and results in another study. Although the reason for the discrepancy is not known, the authors favor the ultracentrifugation purification method over the precipitation method, which has not been tested for use with lipoprotein samples from camels. It is possible that differences in lipid and protein composition could lead to differential interaction with the precipitating reagents.

Interestingly, we also found that the plasma lipoprotein profile of dromedary camels was not identical to that reported for Bactrian camels, despite their closely related ancestry. In Bactrian camels, only minor quantities of HDL (11 mg/dL) were identifiable. However, in both dromedary and Bactrian camels, VLDLs were similarly enriched in triglyceride (58% in both species), and LDL was enriched in cholesterol (37% and 68%, respectively). It has been suggested that Bactrian camels lack a true class of high-density macromolecules and that LDLs and HDLs in that species form a continuum of molecules with a density near the boundary of 1.063 g/ml. Nonetheless, we have not found such a pattern in dromedary camels. High-density lipoprotein is the principle class of plasma lipoprotein in cattle, sheep, and pigs.

The lipoprotein pattern in 1-humped camels differs from that of most other animals, including the closely related Bactrian camels. A large portion of the plasma lipid in Bactrian camels was recovered in the HDL and LDL fractions. Importantly, LDL appears to be the principal carrier of cholesterol in dromedary camels. The results for the study reported here revealed substantial differences from other published data on serum lipoproteins in other animals. Comparison between the lipoprotein profiles obtained from several animal species should lead to a better understanding of the importance of lipid distribution and composition on the pathogenesis of lipoprotein disorders.

References


Correction: Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration

In the report “Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration,” (Am J Vet Res 2008;69:751–762), information in Table 2 on page 756 is incorrect. For the variable Baseline, the unit of measurement is incorrectly listed as mmol/L. The correct unit of measurement is pg/ml.