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Mass spectrometric measurements of the apolipoproteins of bovine (*Bos taurus*) HDL

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ABSTRACT

As is the case in most mammals, high density lipoproteins (HDL) also comprise the major group of lipid carriers that circulate in bovine (*Bos taurus*) blood. As a continuation of our proteogenomic studies of mammalian apolipoproteins, we have obtained molecular masses for several of the apolipoproteins associated with bovine HDL. The major apolipoprotein on the HDL surface is apoA-I, but other apolipoproteins were also detected. Using electrospray-ionization mass spectrometry (ESI-MS), we report on values for apolipoproteins, A-I, proA-I and A-II, as well as post-translationally modified apoA-I. Analyses of tryptic fragments did reveal the presence of apoA-IV and apoC-III. However, in contrast to our previous studies of other mammalian HDL, we did not detect apoC-I. Interestingly, examination of the current assembly for the bovine genome does not show any evidence for an apoC-I gene.

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1. Introduction

Water insoluble lipids, such as triacylglycerol and cholesteryl ester molecules, circulate in the blood by being packaged into spheroidal complexes of lipids and proteins, called lipoproteins. The lipoproteins are polydisperse, varying in size and density, the latter being dependent primarily on the lipid to protein ratio. For most fasting mammals, humans being an exception, the highest concentrations of lipoproteins are in the high density lipoprotein (HDL) class (Chapman, 1980). In humans, the protein moiety of HDL consists of two major apolipoproteins, apoA-I and apoA-II, in an approximate molar ratio of 2 to 1 (Scanu and Edelstein, 2008). In cattle, on the other hand, in which the major lipoprotein class is HDL, the protein moiety of bovine HDL consists primarily of apoA-I with relatively smaller amount of other proteins (Puppione et al., 1982; Bauchart et al., 1989). During lactation, HDL levels in cows become quite elevated (Raphael et al., 1973; Puppione, 1978,1983; Puppione et al., 1980).

The lipids absorbed in the small intestine of cattle are mostly saturated as a result of the biohydrogenating action of the various ruminal microorganisms (Noble, 1978; Puppione, 1983). Nevertheless, a significant amount of polyunsaturated fatty acids are absorbed as indicated by their presence in the core of bovine HDL (Evans et al., 1961; Puppione, 1983). However, in the cores of the less dense lipoproteins the cholesteryl esters are either saturated or monoenoic (Evans et al., 1961). The presence of a high content of polyunsaturated cholesteryl esters in the HDL is consequence of the transesterification reaction catalyzed by lecithin cholesterol acyl transferase (LCAT) (Glomset, 1968; Noble et al., 1972). As a result, the HDL are the major source of essential fatty acids in bovine plasma. The LCAT reaction in which polyunsaturated fatty acids are removed from lecithin and esterified to cholesterol is activated by apoA-I (Scanu and Edelstein, 2008).

Serum concentrations of apoA-I have been measured in cows and have been found to increase during lactation (Mazur et al., 1989). The cDNA of apoA-I has been cloned and sequenced (O'hUigin et al., 1990) and also the apoA-I primary sequence has been determined, revealing it to contain 241 amino acids, two less than human apoA-I (Sparrow et al., 1992). Based on these data the calculated molecular mass corresponds to 27,549.0 Da. However, electrophoretic analyses have reported molecular mass values as low as 26 kDa (Draisci et al., 2007) and as high as 28 kDa (Puppione et al., 1982).

Using mass spectrometry, our laboratory has been involved in proteogenomic examination of the apolipoproteins associated with different mammalian HDL. In addition to obtaining accurate molecular masses we have also reported on various post-translational modifications of apolipoproteins. Because of the importance of HDL in bovine metabolism, we have carried out similar studies on the lipoprotein of cattle. Following chromatographic separation, intact molecular mass values were obtained for apoA-I, proA-I, and apoA-II. In addition, we detected post-translational modifications of both apoA-I and apoA-II. Further analyses by tandem mass spectrometry (MSMS) were done on the tryptic digests of apolipoproteins separated by reverse-phase chromatography, thus providing confirmatory sequences for apoA-I. The analyses of the tryptic fragments also revealed the presence of apoA-II, apoA-IV and apoC-III.

2. Materials and methods

2.1. *Samples collection*

Samples were obtained from eighteen healthy Holstein-Friesian bulls (*Bos taurus*), which were being study to evaluate the effect of administration of dexamethasone. This drug cannot be given to cattle in Italy and other European Union countries for the enhancement of growth. Animals were fed once a day with "Unifeed" consisting of corn silage 9 kg, hay 1.5 kg, nucleus 1.7 kg, cotton 1 kg, maize 5 kg and vitamins and minerals integrator 0.25 kg. In addition, they were divided into three groups based on the administered daily dose of dexamethasone mixed with the feed: group A (0.7 mg); group B (1.4 mg); group C (the control). The animals were maintained in accordance with Italian legislation, Decreto legislativo n.1 16/92 on animal welfare, and approved by Ministry of Health of Italy.

Blood was collected by jugular venipuncture from all the animals into 10 mL evacuated tube, allowed to clot and centrifuged at room temperature for 10min at 2000 rpm (814g) (Gardini et al., 2006). Sera were stored at -80°C in 0.300 mL aliquots until their shipment to the University of California in Los Angeles. Analyses at UCLA were done primarily to obtain accurate molecular mass data on the apoli-poproteins associated with bovine HDL and also to see if possibly there were any modifications of the apolipoprotein mass related to the administration of dexamethasone. Samples obtained after 49 days from the onset of these studies were analyzed. Our data indicated that drug administration had no discernible effect on the apolipoprotein mass.

2.2. *Ultracentrifugal isolation of apolipoproteins*

To obtain representative data of bovine apo HDL 720 μL pools were prepared from each group. Each consisted of 120 μL aliquots of six different animals mixed together. The density of the pool was adjusted to 1.063 g/mL upon the addition of a concentrated NaBr solution (density = 1.489 g/mL). From each pool, 180 μL of the adjusted serum were transferred into 4 polycarbonate tubes and spun in a Beckman Optima-TLX ultracentrifuge using a TLA-100 rotor at 80,000 rpm (250,000 g at r_{av}) at 20°C for 3 h. After removal of 60 μL from the top, the infranatant densities were adjusted to a density of 1.195 g/mL with the addition of a NaBr solution (density = 1.489 g/ mL) and the samples were spun again for 4 h. Then, 40 μL of HDL solution from the top were recovered and a resulting pool of 0.160 mL HDL apolipoproteins was obtained for each group. Using Centricon centrifugal filters with a nominal molecular mass cut off of 10,000 Da (Centricon-10 devices, Millipore), the fractions were adjusted to a density approaching 1.006 g/mL with a dilute NaCl solution. Samples were transferred to Centricon tubes with 2 mL of NaCl solution and centrifuged in a table top centrifuge until the volume was approximately 0.100 mL All salt solutions contained 0.04% $\text{Na}_2\text{-EDTA}$ and 0.05% NaN_3 (Schumaker and Puppione, 1986).

2.3. *Analysis of apolipoproteins by size exclusion chromatography-mass spectrometry SEC-MS*

The protocols of Whitelegge and co-workers were used to obtain molecular masses of the apolipoproteins. Volumes of 0.015 mL of di-alyzed HDL solution were acidified upon addition to 0.1 mL of 90% formic acid. SEC-MS was performed in a degassed solution of CHCl_3 / MeOH/1% aqueous formic acid (4/4/1; v/v/v) using a SW2000 column (4.6x300 mm, Tosoh Bioscience, Montgomeryville, PA, USA) at 0.250 mL/min and 40 °C (Whitelegge et al., 1998). Immediately prior to interacting with the electrospray-ionization source, the column effluent was monitored with a UV detector set at 280 nm. Mass spectrometry (ESI-MS) was performed using a triple quadru-pole instrument (API III, Applied Biosystems) tuned and calibrated as with a flow injection of polypropylene glycol (PPG) monitoring eight signals (the singly charged ions at m/z 58.99, 326.25, 906.67, 1254.92, 1545.13, 1863.34 and 2010.47, and the doubly charged ion at m/z 520.4 (Whitelegge et al., 1999). Data were analyzed using Mac-Spec 3.3 and BioMultiview 1.3.1 software (Applied Biosystems). Using MacSpec 3.3, it was possible to extend the calculations of molecular mass to the first decimal place, except for apoA-II. Values for A-II shown in the text and table and figures were obtained using BioMultiview 1.3.1. The resulting molecular mass values were compared with calculated molecular masses derived from genomic entries in various databases that included the National Center for Biological Information (<http://www.ncbi.nlm.nih.gov>), the Genome Bioinformatics website of the University of California at Santa Cruz (UCSC) (<http://genome.ucsc.edu/>) and the Ensembl website (<http://www.ensembl.org/index.html>). Calculated values for molecular mass were obtained using ProtParam at the proteomic server of the Swiss Institute of Bio-informatic (<http://ca.expasy.org/>).

2.4. Separation of the apolipoproteins by reverse phase column chromatography

Partial delipidation was performed by mixing 0.080 mL of HDL solution with 1 mL of 80% cold acetone and incubated for 30 min at — 20 °C. After a 5 min centrifugation at room temperature, the precipitated apolipoproteins were dried for 5 min in open tubes, dissolved in 0.100 mL of 90% formic acid and injected onto a reverse phase column (PLRP/S 5 mm, 300 Å, 2 x 150 mm, Polymer Labs, Amherst, MA, USA), previously equilibrated in 95% A, 5% B (A, 0.1% trifluoroacetic acid in water; B, 0.05% trifluoroacetic acid in acetonitrile/isopropanol 1:1) and eluted with a compound linear gradient from 5% B at 5 min after injection to 100% B at 60 min (Whitelegge et al., 2002). The column was tested with blank injections of 90% formic acid before every experiment. Column temperature was 40 °C and the flow rate was 0.1 mL/min. Fractions were collected into 2 mL tubes at 1-min intervals concomitant with ESI-MS using a liquid-flow splitter inserted between the HPLC detector and mass spectrometer (LC-MS+). Analysis of the resulting ESI-MS data enabled the correspondence between fraction number and apolipoprotein elution time to be determined.

2.5. Tryptic digestion of apolipoproteins

Fractions collected between 45 and 80 min were selected for further analyses. Prior to tryptic digestion, proteins were reduced and alkylated. Aliquots of 0.010 mL from collected fractions, were incubated with

0.015 mL of 100 mM ammonium bicarbonate and 0.002 mL of 0.01 M DTT for 1 h at 37 °C. After a second hour of incubation with 0.005 mL 0.055 M iodoacetamide, trypsin (Grade Modified Trypsin Porcine, Promega, Madison, WI USA) was added at 37 °C for 3 h. The samples were then stored at — 80 °C until MS/MS analyses were done.

2.6. μ LC-MS/MS analysis

Apolipoproteins in the various fractions were analyzed by μ LC-MSMS with data-dependent acquisition (Q STAR XL, Applied Biosystems, Foster City, CA) after dissolution in 0.010 mL 0.1% formic acid, 5% acetonitrile (v/v). The calibration of the instrument was performed using glufibrinopeptide (GFP, Sigma) in H₂O/ACN 0.1% formic acid solution injected at flow rate of 50 nL/min. Calibration was obtained using the product ions generated from the fragmentation of the doubly charged molecular ion of glufibrinopeptide (500 fmol/ mL in acetonitrile/water 1:1 containing 0.1% v/v formic acid) at m/z 785.888. Nitrogen was used as the collision gas. A reverse-phase column (200 μ m x 10 cm; PLRP/S 5 mm, 300 Å; Michrom Biosciences, San Jose, CA) was equilibrated for 20 min at 0.002 mL/min with 95% A, 5% B (A, 0.1% formic acid, 5% acetonitrile in water; B, 0.1% formic acid in acetonitrile) prior to sample injection (0.005 mL). A compound linear gradient was initiated 3 min after sample injection ramping to 80% A, 20% B at 8 min; 65% A, 35% B at 13 min; 25% A, 75% B at 23 min; 90% A, 10% B at 23.1 min. Column eluent was directed to a stainless steel nano-electrospray emitter (ES301; Proxeon, Odense, Denmark) at 4.4 kV for ionization without nebulizer gas. The mass spectrometer was operated in "IDA" mode (information dependent acquisition) with a survey scan (400-1500 m/z), data-dependent MSMS on the two most abundant ions with exclusion after two MSMS experiments.

Data from MS/MS analyses were processed using the Mascot program and the non-redundant database at the National Center for Biotechnology Information (NCBI). The search parameters were: 1 Da mass tolerance for the parent mass of + 2 and + 3 charge state and 0.6 Da for the fragment masses. The search was run under the "no enzyme" mode. Carbamidomethylation as fixed modifications and methionine oxidation as variable modifications were selected.

3. Results

3.1. *Molecular mass values for bovine apoA-I and A-II*

Following size exclusion chromatography, the molecular masses of apoA-I for each of the three groups were obtained with slightly higher values than the calculated molecular mass, 27,549.08 Da (**P15497**). The observed values were 27,552.9 Da for Group A, 27,551.9 for Group B and 27553.4 for Group C. In each spectrum, a minor peak was observed with a molecular mass of 28,442.7 \pm 4.6 Da. The calculated molecular mass of bovine proapoA-I is 28,432.06 Da (**P15497**). For each group, a major peak was observed for truncated apoA-II with the identical molecular mass value of 8551 Da. The truncation due to the loss of the C-terminal arginine results in a calculated molecular mass of 8549.5 Da (**P81644**).

When analyses were done following reverse-phase chromatography, mass values closer to the calculated

molecular masses for proapoA-I, apoA-I and truncated apoA-II were found (Table 1). Also shown in Table 1 are mass values for acylated apoA-I. A representative spectrum with molecular mass values for proapoA-I, apoA-I and truncated apoA-II is shown in Fig. 1A. Post-translationally modified apoA-I, reflecting acylation, is shown with apoA-I and proapoA-I in Fig. 1B.

3.2. MSMS analyses of apo HDL tryptic fragments

Selected fractions, collected after reverse-phase chromatography and enriched in the various apolipoproteins, were incubated with trypsin as described in Section 2.5 and then analyzed by tandem mass spectrometry. Multiple enzymatic fragments from apoA-I were detected and sequenced, resulting in 76.3% coverage. The resulting data are compared with the complete sequence for mature apoA-I in Table 2. Although not detected by ESI-MS following reverse-phase separation, enzymatic fragments derived from A-IV and C-III

Table 1

Molecular mass values of bovine apolipoproteins detected by ESI-MS.

Bovine Groups	proapoA-I	acylated A-I	apoA-I	truncated A-II
A	28,434.9 Da	27,816.2 Da	27,551.0 Da	8551 Da
B	28,436.4 Da	27,815.7 Da	27,552.8 Da	8551 Da
C	28,436.0 Da	N. D.	27,551.9 Da	8551 Da

For comparison, the molecular weights of proA-I and A-I are 28432.06 Da and 27549.08 Da, respectively (P15497).

The molecular weight of truncated A-II is 8549.5 Da (P81644). The molecular weight of acylated A-I is 27,815 Da, assuming an 18 carbon fatty acid is covalently associated with the apolipoprotein. N.D. Not detected.

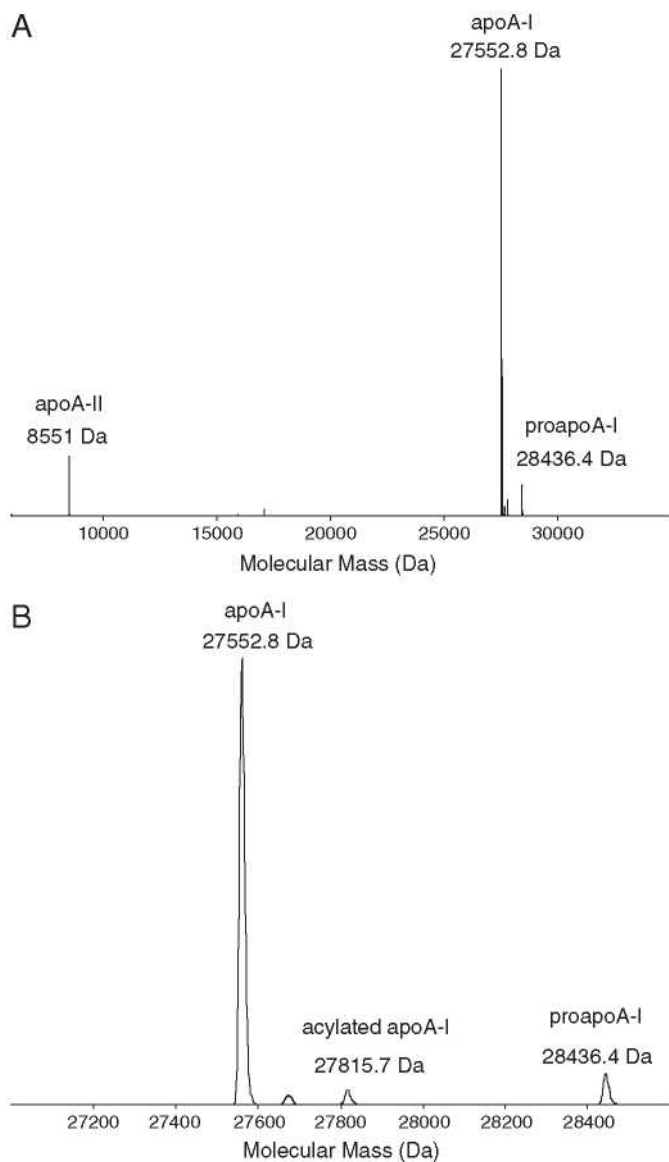


Fig. 1. Electrospray-ionization mass spectral profile of group B apolipoproteins separated by reverse-phase chromatography. (1A) ProapoA-I, apoA-I and truncated apoA-II. (1B) ApoA-I (27,552.8 Da), proapoA-I (28,436.4Da), acylatedapoA-I (27,815.7 Da). Ordinate units of intensity are linear and arbitrary; the abscissa units of average molecular mass are in Daltons.

were analyzed with MSMS. The resulting data and those for apoA-II are shown in Table 3 along with the corresponding primary sequences. In both Tables 2 and 3, the peptides that were detected appear underlined and in bold type.

4. Discussion

In previous studies of lactating cows, we have reported that the lipoproteins enriched in apoA-I are polydisperse, with densities varying throughout the HDL range and extending into the LDL range (Puppione et al., 1982). Carrying out detailed molecular mass measurements on apoA-I in this study, we have also detected other apolipoproteins associated with bovine HDL.

Following size exclusion chromatography, molecular mass values were obtained both for the mature and the pro form of apoA-I. The propeptide can be removed either in the liver or in the circulation (Gordon et al., 1983, 1986). What percentage of circulating proapoA-I is associated with HDL or is present in a separate soluble

<u>DDPQSSWDRV KDFATVYVEA IKDSGRDYVA QFEASALGKQ LNLKLLDNWD</u>		
<u>TLASTLSKVR EQLGPVTQEF</u>		
<u>WDNLEKETAS LRQEMHKDLE EVKQKVQPYL DEFQKKWHEE VEIYRQKVAP</u>		
<u>LGEEFREGAR QKVQELQDKL</u>		
<u>SPLAQELRDR ARAHVETLRQ QLAPYSDDLRLRLTARLEAL KEGGSLAEY</u>		
<u>HAKASEQLKA LGEKAKPVLE</u>		
<u>DLRQGLLPVL ESLKVSILAA IDEASKKLNLA Q</u>		
Residues	Sequences	Mascot score
10-26	<u>R.VKDFATVYVEAIKDSGR.D</u>	89
10-22	<u>R.VKDFATVYVEAIK.D</u>	94
12-26	<u>K.DFATVYVEAIKDSGR.D</u>	84
12-22	<u>K.DFATVYVEAIK.D</u>	55
23-39	<u>K.DSGRDYVAQFEASALGK.Q</u>	89
27-39	<u>R.DYVAQFEASALGK.Q</u>	85
45-58	<u>K.LLDNWDTLASTLSK.V</u>	116
59-82	<u>K.VREQLGPVTQEFWDNLEKETASLR.Q</u>	125
59-76	<u>K.VREQLGPVTQEFWDNLEK.E</u>	117
61-82	<u>R.EQLGPVTQEFWDNLEKETASLR.Q</u>	93
61-76	<u>R.EQLGPVTQEFWDNLEK.E</u>	82
96-105	<u>K.VQPYLDEFQKK</u>	43
96-106	<u>K.VQPYLDEFQKK.W</u>	49
107-115	<u>K.WHEEVEIYR.Q</u>	69
118-130	<u>K.VAPLGEEFREGAR.Q</u>	55
118-126	<u>K.VAPLGEEFR.E</u>	64
131-150	<u>R.QKVQELQDKLSPLAQELRDR.A</u>	53
131-148	<u>R.QKVQELQDKLSPLAQELR.D</u>	49
133-148	<u>R.QKVQELQDKLSPLAQELR.D</u>	108
160-172	<u>R.QQLAPYSDDLRLR.L</u>	27
160-170	<u>R.QQLAPYSDDLRLR.Q</u>	70
177-193	<u>R.LEALKEGGSLAEYHAK.A</u>	78
205-224	<u>K.AKPVLEDLRLRQGLLPVLESILK.V</u>	82
205-213	<u>K.AKPVLEDLRLR.Q</u>	47
214-224	<u>R.QGLLPVLESILK.V</u>	67
225-236	<u>K.VSILAAIDEASKK</u>	92
225-237	<u>K.VSILAAIDEASKK.L</u>	87

The 241 amino acid sequence of mature bovine apoA-I is shown at the top of the table (P15497). Tryptic peptides detected are underlined and in bold type.

Table 2

Tryptic fragments of bovine apoA-I detected by tandem mass spectrometry.

apoA-II (P81644)				
QAEESNL QSLVSQYFQT VADYGKDLVE KAKGSELQTO				
AKAYFEK TQE ELTPFFKKAG TDLNLFSSF IDPK KQPATR				
Mascot scores were 58 for fragment T45 to K54 and 48 for fragment A56 to K71.				
apoA-IV (Q32PJ2)				
EVNADQVATV	IWDYFSQLGN	NAKKAVEHIQ	KSELTQQLNT	LFQDKLGEVS
TYTDDLQKKL	VPPATELHER	LTKDSEKLKE	EIRKELEDLR	ARLLPHATEV
SQKIGDNRVE	LQQRGLPYAE	ELRTOVDTOA	QQLRRQLTPY	VERMEKVMRQ
NLDQLQASLA				
PYAEELQATV	NQRVEELKGR	LTPYADQLQT	KIEENVEELR	RSLAPYAQDV
QGKLNHQLEG	LAFQMKKHAE	ELKAKISAKA	EELRQGLVPL	VNSVHGSQLG
NAEDLQKSLA	ELSSRLDQQV			
EDFRRTVGPY	GETFNKAMVQ	QLDTRLRQKLG	PLAGDVEDHL	SFLEKDLRDK
VSSFFNTLKE	KESQAPALPA	QEEMPVPLGG		
Mascot scores for the following fragments were: 68 for E1 to K23, 90 for S32 to K45, 72 for L46 to K58, 49 for L46 to K59, 20 for K59 to R70, 63 for L115 to R123, 89 for Q150 to R173, 40 for L181 to K191, 70 for S202 to K213, 65 for Q245 to K267, 46 for T286 to K296, 71 for A297 to R306, 47 for L309 to K325.				
apoC-III (P19035)				
EEGSLLD KMQGYVKEAT KTAKDALSSV QESQVAQQAR				
DWMTESFSSL KDYWSSFKGK FTDFWESATS PTQSPP				
Mascot scores were 68 for fragment T19 to R37, 122 for fragment D22 to R37 and 26 for fragment D49 to K55.				

Tryptic peptides detected are underlined and in bold type. The C-terminal arginine is shown in red to indicate that it is the missing amino acid in truncated apoA-II.

Table 3

Sequences of minor apolipoproteins on bovine HDL with the various tryptic fragments detected by

complex with or without lipids has never been determined. The propeptide is a hexapeptide, RHFQQ (P15497). With the addition of these six amino acids, bovine proapoA-I has a calculated molecular mass of 28,432.06 Da. Our data for each of these apolipoproteins were in agreement with molecular weights calculated from primary sequences determined from entries in genomic databases. Previously, Motizuki et al. had reported that bovine apoA-II had 76 amino acids (Motizuki et al., 1998). The genomic entry (P81644) for apoA-II encodes a 77 amino acid apolipoprotein with a sequence agreeing with that reported by Motizuki et al. except for an additional arginine at the C-terminus. Our molecular mass value for apoA-II is consistent with a 77 amino acid protein.

Molecular mass values obtained after the apolipoproteins were separated by reverse-phase chromatography were essentially the same for these two apolipoproteins. In previous studies of various mammalian HDL, we have reported that a small percentage of apoA-I is acylated (Puppione et al., 2005, 2006, 2008, 2009). Bovine apoA-I undergoes a similar posttranslational modification (Fig. 1B). Tryptic digestion on selected fractions that were collected during the run enabled "Bottom Up" sequencing to be done on apoA-I. As the data in Table 2 indicate, this resulted in 76.3% coverage when compared to the previously reported apoA-I sequence. These analyses also demonstrated the presence of other apolipoproteins, viz. apoA-II, apoA-IV and apoC-III.

In characterizing the molecular masses of diverse mammalian apolipoproteins, we have reported that apoC-I

was present on HDL (Puppione et al., 2006, 2008, 2010). This was not the case in this current study. However, Brzowska et al. had reported that the gene for bovine apoC-I was located in an apolipoprotein gene cluster on chromosome 18 (Brzowska et al., 2000). Although an examination of the database does reveal three apolipoprotein genes in a 19.3 kb cluster on this chromosome, the most recent assembly (October, 2007) for the bovine genome does not contain an entry for apoC-I. The coordinates on chromosome 18 for the three genes were: 52423183 to 52425869 for apoE, 52437317-52439700 for apoC-IV and 52440256-52442497 for apoC-II. In other mammalian studies, the apoC-I gene has been shown to be present between the genes for apoE and apoC-IV. In the interval between 52438700 and 52425869, there were no sequences corresponding to mammalian exons of apoC-I.

One of the many putative roles for apoC-I is being an inhibitor to the enzyme, cholesteryl ester transfer protein (CETP) (Gautier et al., 2000). Even though it appears that this apolipoprotein is absent in cattle, it is interesting to note that bovine plasma also contains low levels of CETP (Ha and Barter, 1982). This would explain why bovine HDL are highly enriched in polyunsaturated cholesteryl esters whereas the cholesteryl esters in the less dense lipoproteins contain saturated fatty acids (Evans et al., 1961). CETP enables the exchange of cholesteryl esters among the different lipoprotein classes. There are two major sites of synthesis of cholesteryl esters, the liver and the HDL in plasma. Whereas hepatocytes synthesize saturated and mono-enoic cholesteryl esters, those produced in the plasma through the action of LCAT are primarily polyunsaturated. The selective partitioning of cholesteryl esters among bovine lipoproteins may have relevance to ongoing clinical trials evaluating the efficacy of CETP inhibitors. These trials are testing whether the retention of cholesteryl esters in the HDL, thereby increasing the levels of HDL cholesterol, will be beneficial to cardiovascular health (Davidson, 2010). However, by preventing the movement of polyunsaturated cholesteryl esters to the less dense lipoproteins, the LDL will then have a core of saturated cholesteryl esters with a much higher melting point than normal. Besides just focusing on the potential benefits of higher HDL levels, it might be wise for these trials first to determine if CETP inhibition alters how the various fatty acids esterified to cholesterol are distributed among the lipoprotein classes. This is not a problem in cattle that have both a shorter life span and much lower levels of LDL in their circulation than humans. However, the higher levels of human LDL with a core of saturated cholesteryl esters may cause a problem.

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