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EVIDENCE FOR THE PRESENCE OF LOW DENSITY AND VERY LOW DENSITY LIPOPROTEINS IN HUMAN AMNIOTIC FLUID

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Summary: Previous analysis of amniotic fluid (AF) noted only the presence of high density lipoprotein (HDL). In this study AF lipoprotein profile was examined using gel filtration column chromatography and Ouchterlony gel diffusion. Unlike previous studies which showed only the presence of HDL, we found significant amounts of low density lipoprotein (LDL) and very low density lipoprotein (VLDL). AF-LDL and AF-VLDL were identified by reactions with anti-h-apolipoprotein AI and AII antiserum and anti-h-apolipoprotein B-antiserum, respectively. Furthermore, bulk of the cholesterol mass was carried in VLDL (53.6 ± 7.7%) and LDL (32.5 ± 4.3%) with minor amounts (13.9 ± 1.3%) in HDL fraction. It is concluded that human AF contains all three lipoproteins with most of the cholesterol being carried in very low density lipoprotein fraction.

AF has been used for prenatal diagnosis of a variety of metabolic disorders (1-5). However very little application of AF composition has been used in lipoprotein disorders. This is mainly because detailed analysis of AF lipoprotein has not been done. Guibaud, et al., (6) found some α1-lipoprotein in 5 of 8 samples of human AF of unspecified gestational ages, but noted the absence of β-lipoprotein. Gebhardt and Beintema (7,8) showed the HDL profile of AF samples from pregnancies older than 20 weeks. Nencioni, et al., (9) could not detect any β-lipoproteins in AF samples from 36 to 40 week pregnancies. It is not known why human AF from various gestation ages contain only single lipoprotein, HDL, while plasma contains both LDL and VLDL in addition to HDL. In continuation of our previous studies to characterize AF factors influencing lipid metabolism in fetal life (10), we considered it of importance to identify AF-lipoprotein profile and AF-apolipoproteins on immunodiffusion.

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Materials and Methods

Human AF samples were obtained at delivery from subjects of normal pregnancy. For isolation and lipid analysis of the lipoproteins, the AF samples were concentrated 10 times using aquacide 1-A (Calbiochem). For isolation of lipoprotein, density of AF was adjusted to 1.25 mg/ml and was overlayed with KBr soln (d = 1.25) in cellulose-nitrate tubes (12 ml capacity), then centrifuged for 40 hours at 38,000 rpm at 10°C in a Beckman Ultracentrifuge (Beckman, Fullerton, CA). After spinning, the top 1 ml fractions containing AF-lipoproteins were removed by pipette with careful handling. Two ml aliquots of the concentrated lipoprotein samples were incubated for 30 minutes at room temperature with a trace amount of (^{14}C)-cholesterol as a marker. A column (1.5 x 100 cm) packed with Bio-Gel A-5M (Bio-Rad Laboratories, Richmond, California) was used to separate the AF-lipoproteins as described previously (11). The system was operated under gravity with a flow rate of 10 ml/h. The column was equilibrated with 0.15 M NaCl, 0.01% EDTA, pH 7.4 for 24 hours before use.

Fractions containing lipoproteins were concentrated using aquacide 1-A and dialyzed against 0.15 M NaCl, 0.01% EDTA, pH 7.4 prior to lipid analysis. The concentration of cholesteryl ester was determined by GLC method as described previously (12). Triglyceride concentrations were measured enzymatically in our Lipid Research Clinic, and protein concentrations were determined by the method of Bradford (13).

For qualitative analysis of the apolipoprotein composition, AF-lipoprotein peaks pooled from column chromatography were subjected to Ouchterlony's immunodiffusion test (14). Three lipoprotein peaks and anti-h-apolipoprotein AI, AII and B-antiserum were applied in holes on the gel plates. Plates were incubated at 37°C until visible bands of precipitation are formed.

Results

AF lipoprotein profile obtained on gel chromatography as shown in Figure 1 clearly shows 3 different lipoprotein species. As compared with human serum, AF lipoproteins eluted at almost identical position as HDL,

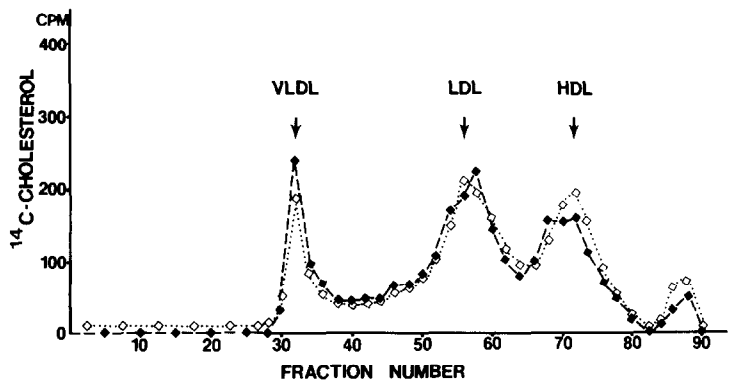

Figure 1. Molecular-sieve chromatography pattern of human serum lipoprotein (··◇··) and AF-LPs (-◆-) on a Bio-Gel A-5M column (1.5 x 100 cm). Radioactivity was monitored as a marker. The elution positions of major lipoproteins are indicated.

TABLE I

FREE AND ESTERIFIED CHOLESTEROL CONTENT OF WHOLE AF AND AF-LIPOPROTEINS (MEAN ± SEM, N=7)

FRACTION	CHOLESTEROL CONTENT (ug/dl AF) FREE	ESTER	TOTAL	CE (%)
WHOLE AF	640 ± 120	761 ± 130	1401 ± 144	52.6 ± 5.8
AF-LIPOPROTEINS				
VLDL	500 ± 87	250 ± 31	950 ± 59 (53.6 ± 4.7%)	33.9 ± 2.2
LDL	200 ± 35	260 ± 29	460 ± 38 (32.5 ± 4.3%)	57.1 ± 4.1
HDL	60 ± 15	140 ± 17	200 ± 18 (13.9 ± 31.%)	71.8 ± 4.8

LDL and VLDL of human serum lipoproteins when subjected to gel chromatography. Furthermore, total cholesterol analysis of the lipoprotein fractions indicated that cholesterol was mainly carried in VLDL and LDL fractions with low amount present in HDL fraction (Table 1). However, esterified cholesterol was mainly present in HDL fraction (71.8%). Non-lipoprotein peak shown in Figure 1 was also present in human serum lipoprotein which is believed to be a non-lipoprotein lipid complex. The identity of the lipoproteins was also confirmed with immunodiffusion (Figure 2). Total contents of protein, and cholesterol in AF were 1196.1 ± 20 mg/dl, and 1400 ± 144 ug/dl (Table 1).

Discussion

Study by Gebhardt, et al., (8) reported that human AF contains only high density lipoprotein particles and had two components on crossed immunoelectrophoresis (the faster and the slower moving HDL components). These authors could not detect the presence of either low or very low density lipoproteins. Using concentrated AF, we have demonstrated for the first time that human AF contains three distinct lipoproteins: VLDL, LDL and HDL. Apolipoproteins AI, AII and B from AF were detected on

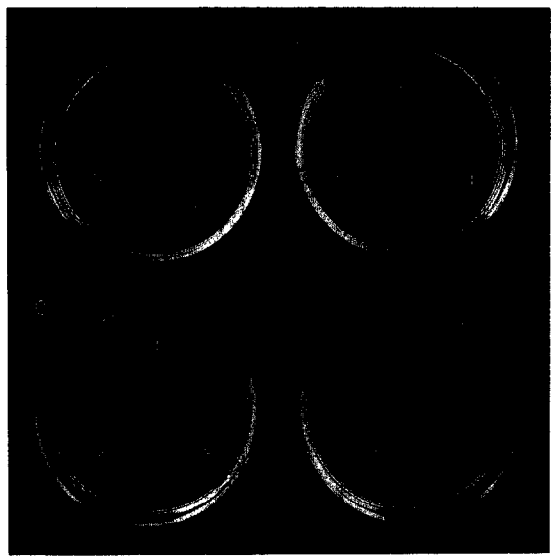

Figure 2. Immunodiffusion of AF-lipoprotein preparations (A,B, and C) and whole AF (D) against anti-apolipoproteins. Wells labeled contained: Plate A; (1) anti-apolipoprotein AI, (2) AF-HDL peak, (3) AF-LDL peak, (4) AF-VLDL peak, plate B; (1) anti-apolipoprotein AII, (2) AF-HDL peak, (3) AF-LDL peak, (4) AF-VLDL peak, plate C; (1) anti-apolipoprotein B, (2) AF-HDL peak, (3) AF-LDL peak, (4) AF-VLDL peak, plate D; (1) whole AF, (2) anti-apolipoprotein AI, (3) anti-apolipoprotein AII, (4) anti-apolipoprotein B.

immunodiffusion. The presence of apo B strongly indicates the presence of LDL and VLDL. On the basis of cholesterol content VLDL and LDL accounted for about 85% of total lipoproteins. Our recent studies with rabbit AF also strongly show the presence of lower density lipoprotein (unpublished data).

The source of the AF lipoprotein is not yet clear (14). The proportion of cholesterol in the AF lipoprotein species is not similar to that from human plasma lipoproteins suggesting that AF lipoproteins could be modified from maternal or fetal lipoproteins or newly synthesized in placenta. As shown in our Ouchterlony immunodiffusion, (Figure 2) apo B protein was detected in both whole AF and AF-VLDL and AF-LDL. Percentage of AF cholesteryl ester was much lower than that of serum (52.6 $\pm$ 5.8% in AF, 65 to 80% in serum) indicating that lecitin:cholesterol acyltransferase (an enzyme which is secreted from liver and esterifies free cholesterol to cholesteryl ester in circulation) is deficient in AF. Lower

percentages of esterified cholesterol found VLDL and LDL (Table 1) also suggest that CETP (cholesterol ester transfer protein) is deficient in AF. However, origin of AF-HDL is believed to be fetal/adult lung or serum (8). Casu, et al., (16) identified a low molecular weight lipopeptidic fraction which was present only in AF and could never be detected in maternal or cord blood serum of the same individuals from whom the AF samples were taken. In the light of the present results, it is unlikely that there is only single lipoprotein in the AF throughout pregnancy. However the origin of AF lipoprotein and maternal contribution during pregnancy deserved further investigation.

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