Vol. 95, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 31, 1980 Pages 738-744

SERUM LIPOPROTEINS AND ALBUMIN IN THE LECITHIN:CHOLESTEROL ACYLTRANSFERASE REACTION

Jeffrey K. Yao, Steve C.S. Chang, Robert J. Ryan and Peter James Dyck

Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received May 31,1980

The unique features of pig ovarian follicular fluids, i.e., presence of high density lipoprotein (HDL) only and lecithin:cholesterol acyltransferase (EC 2.3.1.43; LCAT) activity, provides a good model to study the effect of serum lipoproteins and serum albumin on the LCAT reaction. In vitro cholesterol esterification is enhanced when very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fractions are added, but is inhibited when one or the other of these lipoproteins is absent. High concentrations of HDL_2 result in decreased activation which can be compensated for by the addition of the VLDL-LDL mixture. These findings suggest that the rate of cholesterol esterification in ovarian follicular fluid may be enhanced by providing the exogenous VLDL and LDL as the recipients of HDL-cholesteryl ester. The inhibition of LCAT activity caused by free fatty acid and lysophosphatidylcholine can be partially reversed by the addition of serum albumin, suggesting that serum albumin may regulate the LCAT reaction.

Lecithin:cholesterol acyltransferase (EC 2.3.1.43; LCAT) is involved in the biosynthesis of plasma (or serum) cholesteryl esters in many species (1). Although little is known about the physiological importance of LCAT and its relationship to plasma lipoproteins, a deficiency of this enzyme, as seen in patients with familial LCAT deficiency (2,3) or with Tangier disease (4,5), results in serious metabolic dysfunction. Earlier studies have shown that the LCAT activity depends upon the functional state of the liver and the quantity and lipid composition of the lipoprotein substrate (1,6,7). Abnormal activity is often found in patients having abnormal plasma lipoprotein profiles such as in hypertriglyceridemia, hypercholesterolemia, hyperlipidemia and hyperlipoproteinemia.

Because of a "blood-follicular barrier" (8), follicular fluid contains only high density lipoproteins (HDL) (8,9). It is thought that the LCAT reaction takes place on or within HDL molecules (10,11). Pig ovarian follicular fluid (POFF) has the ability to esterify cholesterol as we have shown previously (12). Because POFF has these unique features (i.e., presence of

0006-291X/80/140738-07$01.00/0

HDL only and a great deal of LCAT ACTIVITY), it provides a good model to study the LCAT reaction. The purpose of this study was to investigate the effect of various serum lipoproteins and serum albumin on LCAT activity using POFF as a model.

MATERIALS AND METHODS

Pools of pig ovarian follicular fluid (POFF) and pig serum (PS) were collected according to the procedure reported previously (9). Porcine ovaries were removed and stored under ice. Follicles of all sizes were aspirated from batches of 100-150 ovaries and the fluid was pooled and kept on ice. All subsequent steps were performed at 4°C. The pooled follicular fluid was centrifuged at 15,000 x g for 15 min to remove cells and debris. One ml of 1% EDTA was then added to every 100 ml of supernatant. The density of the above solution was adjusted to 1.210 with KBr by the method of Havel et al. (13). The fluid was then centrifuged at 225,000 x g for 18 hours using a 60Ti rotor and a Beckman Model L65 ultracentrifuge. After centrifugation, the top 2 ml of the solution was aspirated from the centrifuge tube and dialyzed overnight against normal saline containing 0.1% EDTA. HDL was identified by paper electrophoresis (14) using oil red EGN for staining.

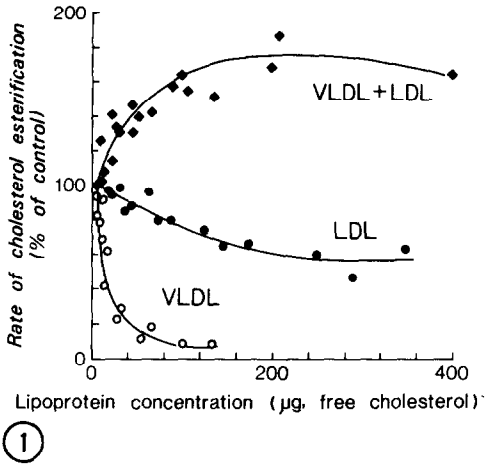
Approximately 100 to 150 ml of pig serum was used for isolation of the lipoproteins. The serum was first centrifuged at 15,000 x g for 10 minutes to remove the chylomicrons. EDTA was added to the serum to make a final concentration of 0.1% before ultracentrifugation. The method used to separate serum into very low density lipoproteins (VLDL, $d \leqslant 1.007$), low density lipoproteins (LDL, $1.007 < d \leqslant 1.065$), combination of VLDL-LDL ($d \leqslant 1.065$), and high density lipoproteins (HDL_2, $1.065 < d \leqslant 1.125$ and HDL_3, $1.125 < d \leqslant 1.210$) was essentially the same as described by Bilheimer et al (15). All the fractions were dialyzed and identified by paper electrophoresis (14).

The initial rate of cholesterol esterification in POFF and PS was determined by the method of Yao et al. (12). [4-^{14}C]Cholesterol (specific activity, 56 mCi/M, Amersham/Searle), which was dissolved in 95% ethanol containing 0.1% of Tween 20, served as a precursor. The triacylglycerol, palmitic acid and lysophosphatidylcholine were dispersed into the above medium if they were required for the experiments. The ethanol was evaporated under nitrogen before POFF, PS, lipoproteins or bovine serum albumin (BSA) were added. The incubation times for POFF and PS were 4 h and 5 h, respectively (12), or as indicated.

The free and esterified cholesterol were extracted from POFF and PS by the method of Peter and Reynolds (16) and quantitated by gas-liquid chromatography (GLC) (17). The activity of LCAT was expressed as either the percentage of labeled cholesterol esterified per unit time (fractional rate) or the number of nanomoles of cholesterol esterified per milliliter of sample per hour of incubation (absolute rate).

RESULTS AND DISCUSSION

When aliquots of VLDL containing various amounts of free cholesterol were added to the POFF incubation medium, the *in vitro* rate of POFF-cholesterol esterification was greatly reduced (Fig. 1). This was also true for the



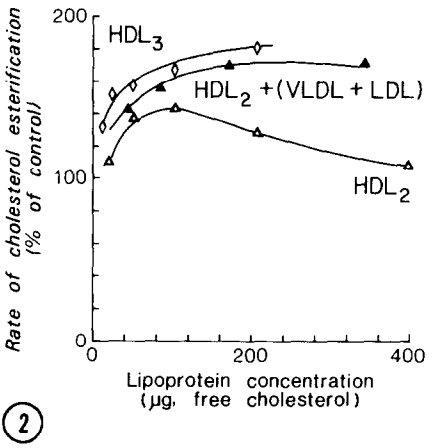


Fig. 1 Effect of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) on the POFF-cholesterol esterification. Each incubation mixture consisted of: 0.5 ml of pig ovarian follicular fluid (POFF), 0.05 μCi of [4-^{14}C]cholesterol, 5 μl Tween 20 and pig serum lipoprotein subfractions (VLDL or LDL) which contained increasing amounts of unesterified cholesterol. The concentration of unesterified cholesterol in POFF, VLDL-LDL ($d \leqslant 1.065$), VLDL and LDL fractions were 45, 993, 81 and 621 μg/ml, respectively. The added lipoprotein substrates were also included in the calculation of specific activity in each incubation. The incubation mixture without addition of lipoproteins served as a control. The absolute rate of esterification for control was 7.50 nmoles/ml/hr.

Fig. 2 Effect of high density lipoprotein subfractions (HDL_2 and HDL_3) and VLDL-LDL combined fraction on POFF-cholesterol esterification. The incubation mixture consisted of: 0.5 ml of POFF, 0.05 μCi [4-^{14}C]cholesterol, 5 μl Tween 20 and lipoprotein fractions (HDL_2, HDL_3 or VLDL-LDL) which contained increasing amounts of unesterified cholesterol. Both HDL_2 and HDL_3 were deactivated at 60° for 30 minutes before adding to the incubation medium. The concentration of unesterified cholesterol in POFF, HDL_2, HDL_3 and VLDL-LDL-HDL_2 ($d \leqslant 1.125$) fractions were 46, 1040, 260 and 860 μg/ml, respectively. Each point is the mean of the two determinations. The absolute rate of esterification for the control was 6.80 nmoles/ml/hr.

addition of LDL to POFF, but to a lesser extent. On the other hand, the addition of a mixture of VLDL and LDL to POFF enhanced the rate of POFF-cholesterol esterification (Fig. 1). This finding was further supported by the results that addition of VLDL and LDL mixture can prevent the suppression of LCAT-activation seen with high concentration of HDL_2 (Fig. 2), and additional VLDL and LDL from heat-inactivated pig serum have the ability to enhance the rate of POFF-cholesterol esterification (Table I). However, the exact reason why the VLDL-LDL mixture causes stimulation of LCAT reaction in POFF is not clear at this time.

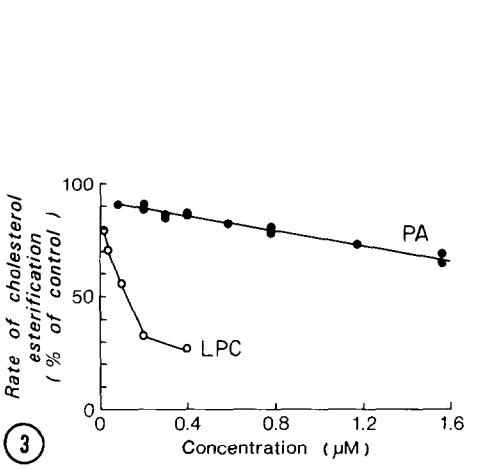
TABLE I. Effect of Pig Serum (PS) on Cholesterol Esterification of Pig Ovarian Follicular Fluid (POFF)

Source of substrate		Substrate Concentration	Rate of cholesterol esterification		
Fresh	Heat-Inactivated		Fractional	Absolute	Change
		g FC[a]	%[b]	nmoles[b]	%
0.5 ml POFF	--	23	28.04[b]	16.62	-
0.5 ml POFF	0.5 ml PS	114	8.70	25.56	+53.8
0.5 ml PS	--	91	31.21	73.20	-
0.5 ml PS	0.5 ml POFF	114	17.86	52.48	-28.3

[a] FC = Free cholesterol
[b] Incubation time was 4 h
[c] Mean value of three determinations

The nonenzymic transfer of triacylglycerol from VLDL to HDL has been demonstrated by incubating human serum at 37°C (18). A corresponding transfer of cholesteryl esters from HDL to VLDL is also accompanied by the exchange of triacylglycerol (18). The LCAT enzyme can enhance the exchange of cholesteryl esters between HDL and VLDL (18,19). On the other hand, the catabolism of VLDL to remove triacylglycerol may require the action of both lipoprotein lipase and LCAT (20). Recently, Rose (21) has demonstrated that LCAT-generated cholesteryl esters are capable of equilibration among lipoproteins. Chajek and Fielding (22) have further shown that human plasma contains an apolipoprotein which mediates the transport of cholesteryl ester from HDL to VLDL or LDL. The rate of cholesteryl ester transport is comparable to the rate of cholesterol esterification by LCAT (22). Knowning that POFF is essentially free of LDL and VLDL, it is not unlikely that the rate of POFF-cholesterol esterification can be enhanced by providing the exogenous lipoproteins (LDL and VLDL) as the recipients of cholesteryl esters from HDL. The removal of cholesteryl esters from HDL may be important for the rate of cholesterol esterification.

The *in vitro* rate of POFF-cholesterol esterification was progressively decreased by *in vitro* addition of increasing amount of lysophosphatidylcho-



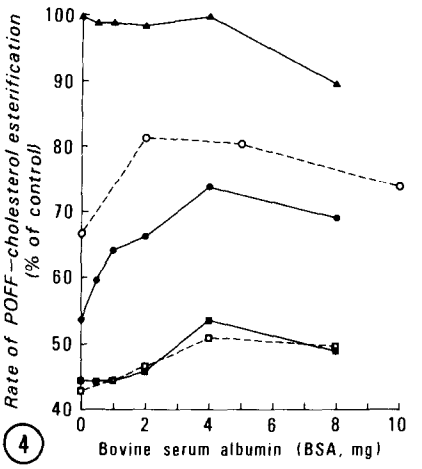


Fig. 3 Inhibition of POFF-cholesterol esterification by in vitro addition of palmitic acid (PA) and of lysophosphatidylcholine(LPC)

Fig. 4 The effects of bovine serum albumin (BSA), palmitic acid (PA) and lysophosphatidylcholine (LPC) on POFF-cholesterol esterification. The incubation mixtures contained either no additions (▲) or 1.56 μM PA (o), 2.34 μM PA (●), 0.2 μM LPC (□ and ■) per 0.5 ml of POFF. The open signs represent experiments where BSA (32 mg/ml) was added in increasing volumes and thus changed the final assay volume. The closed signs represent incubation mixtures in which the concentration of BSA was altered without a change in assay volume.

line to the POFF incubation medium and, to a lesser extent, by palmitic acid (Fig. 3). The inhibition caused by lysophosphatidylcholine and palmitic acid could be reversed partially by addition of increasing amount of BSA (Fig. 4). The experimental condition was not effected by providing various concentrations of BSA in the form of increasing volume or fixed volume (Fig. 4). Incubation of POFF with BSA alone did not cause any decrease in enzyme activity if BSA concentrations were under 4 mg/0.5 ml POFF. Approximately a 10% decrease in LCAT activity was found when up to 16 mg BSA was added to 0.5 ml POFF. This slight inhibition may be due to the contaminations of free fatty acid and other lipids in the commercial BSA.

In addition to HDL, albumin is also present in the ovarian follicular fluid (8). It is known that serum albumin only binds free fatty acids and lysophosphatidylcholine among other lipid fractions. Using POFF as a model, we also confirmed the earlier findings of Rutenberg et al (23) that the inhibition caused by unbound fatty acids and lysophosphatidylcholine could be

reversed partially by *in vitro* addition of bovine serum albumin. Therefore, serum albumin plays an important role in the regulation of the LCAT reaction. In the case of diabetic patients who have decreased LCAT activity (24), their serum cholesterol esterification, at least in part, was inhibited by the large excess of circulating free fatty acids which are known to occur. On the other hand, lysophosphatidylcholine, being a product of the LCAT reaction, may lose its binding site on serum albumin due to the competition of free fatty acids; presumably there are more binding sites for free fatty acids than for lysophosphatidylcholine per mole of serum albumin (23). More recently, Albers et al (25) have shown that, using purified LCAT, albumin can enhance the LCAT reaction in the presence of Apo A-I, possibly by entrapping cholesteryl ester in addition to binding lysophosphatidylcholine.

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