LECITHIN CHOLESTERYL ACYL TRANSFERASE (LCAT) AND HUMAN SERUM CHOLESTEROL LEVELS

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ABSTRACT

From a study of the clinical serum samples obtained routinely and normal serum from several human subjects, it is shown that in hypercholesteremia, the increase in serum cholesterol is largely in the proportion of esterified cholesterol. Using human serum as the combined source of enzyme and substrate, about 50% esterification of the pre-existing cholesterol was observed in 3 hours in normals. With different serum samples of varying cholesterol content, it was observed that the LCAT activity increases with increased serum cholesterol levels.

INTRODUCTION

IN VITRO esterification of cholesterol, on incubation of human serum by a heat-labile enzyme present in serum has long been established by Sperry[1] and several investigations that followed confirmed this finding (D. S. Goodman[2]). That this esterification is mediated through the enzymatic transfer of a fatty acid from the β-position of lecithin to cholesterol by an acyl-transferring enzyme, lecithin cholesteryl acyl transferase (LCAT) has been shown by Glomset[3,4]. Recently it has been reported (D. P. Jones *et al.*[5]) that in certain liver diseases the LCAT activity in serum is depressed, correlating with the percentage and concentration of cholesterol esters found in serum. However, Rutenberg and Soloff[6] have reported that serum LCAT might play an important role in promoting the removal of free cholesterol from the arterial wall while Glomset[4] has discussed the possibility, as a working hypothesis, that LCAT acting intravascularly is responsible for the synthesis of serum cholesterol esters.

The present report is concerned with a study of the role of serum LCAT in controlling and regulating serum cholesterol levels since the degree of conversion of free cholesterol to ester may be of great physiological significance in determining the deposition and penetration of cholesterol and its esters, in lipoprotein complex, on the intima of the arterial wall.

METHODS AND RESULTS

Subjects :

The human serum samples used in this study were obtained from the Medical College Hospital, Alleppey, in the form of routine clinical investigation blood samples. Venous blood withdrawn in the morning before breakfast was immediately chilled, allowed to clot, centrifuged, and serum stored in marked sterile bottles in the freezer till use.

For normal samples, medical students (male), of age between 18–25, acted as volunteers. The hypercholesteremic samples were all from patients referred to our department and suspected clinically of coronary heart disease or acute myocardial infarction.

Cholesterol determination and separation of free and esterified cholesterol by alumina chromatography. —The serum was treated with alcohol-petroleum ether (60–80° C) mixture (1 : 2 v/v), extracted twice, evaporated under nitrogen or under suction and passed through a 3" × ½" column of alumina; the ester was first eluted with 15 ml of 4% acetone in petroleum ether and the free cholesterol was eluted subsequently with 15 ml of 8% ethanol in petroleum-ether. Cholesterol was determined colorimetrically using a spectronic 20 spectrophotometer with the modified Leiberman Burchard reagent[7].

Relative distribution of free and esterified cholesterol in normal and hypercholesteremic serum. —Cholesterol in human serum exists largely (60 to 70%) in the esterified form in normal subjects. In Table I is given a summary of our findings regarding the distribution profile of free and esterified cholesterol in the serum of control subjects (volunteers) and in the hypercholesteremic serum. It is interesting to see that in hypercholesteremia (*i.e.*, total serum concentration above 300 mg%), the increase in the serum cholesterol is more pronounced in the esterified component.

LCAT activity in relation to the pre-existing concentration of cholesterol in serum used as enzymes.—0·25 ml samples of serum obtained from normal or the hypercholesteremic specimens were used as enzymes. These samples (aliquots of 0·5 ml each) were previously extracted and analysed for total, ester and free cholesterol to give the necessary data regarding the cholesterol status of the serum samples used as the enzyme source. In Table II, are summarised the results of the

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TABLE I

Free and esterified cholesterol proportion in normal and hypercholesteremic, human sera

Sample	No. of samples and range	Total cholesterol mg%	Free cholesterol		Esterified cholesterol	
			mg %	% of total	mg %	% of total
Normals	9 (88 –112)	100±10	40±5	40	60±5	60
	9 (140–162)	15±08	60±6	40	90±4	60
	9 (188–212)	20±07	82±4	41	118±7	59
	8 (240–265)	250±10	95±7	38	155±8	62
Hypercholesteremic	19 (280–320)	300±15	100±8	34	200±7	66
	19 (335–362)	350±10	105±5	30	245±6	70
	19 (370–392)	380±10	105±8	27	275±5	72
	16 (420–445)	430±20	108±6	25	322±6	75

Details as given in text. Total 108 samples were analysed, of which 35 were in the normal range and the rest were in hypercholesteremic range. The final values were the mean of the values at that particular range, with S. D.

TABLE II

LCAT activity-time relationship in human sera with different cholesterol content

Sample		mg%		% esterification of pre-existing cholesterol in the heated pooled sera							
		Ester of the enzyme sample	Free of the enzyme sample	Time (Min) incubation							
				zero	30	60	90	120	150	180	
Normal	A	90	60	0	12	23	28	40	43	46	50% (Mean) at 180
	B	118	82	0	14	25	30	45	50	50	
	C	155	95	0	15	26	32	48	50	52	
	D	245	105	0	15	26	31	46	48	50	
Hypercholesteremic	E	320	110	0	16	28	35	52	55	59	60% (Mean) at 180
	F	350	115	0	18	30	35	53	58	60	
	G	350	110	0	17	31	37	48	56	62	

Only typical experiments included. Enzyme assay as given in text. Enzyme source (0·25 ml) of serum, both normal (A to D) and hypercholesteremic (E to G). Substrate was heated pooled serum (4 ml + 6 ml buffer at pH 7·4 for each incubation) from healthy volunteers. Results expressed as mg % in sera of the free cholesterol esterified. Pre-existing levels of cholesterol in the heated substrate serum were 202 mg %, Free, 84 mg % and esters 118 mg %.

study of the *in vitro* esterification by normal human serum (A to D) and of hypercholesteremic serum (E to G). In these studies heat inactivated serum, obtained from human volunteers, were used as the substrate. Four ml serum, diluted with 6 ml buffer (Phosphate pH 7·4, 0·1 M), were heated in a water-bath at 80° C for 10 minutes, cooled to room temperature and the enzyme (0·25 ml) from suitable serum samples added and were incubated at 37° C. One ml aliquots were withdrawn at given time intervals into 1 ml ethanol for stopping the reaction and the free and esterified cholesterol determined. It may be seen that with the normal sera within 3 hrs about 50% of esterification of the pre-existing cholesterol was obtained. This is in agreement with the report of Portman and Sugano[8]. Incubations with different buffers in another series of experiments, indicated that the pH optimum of the serum esterifying system was at 7·4 in PO_4 buffer. However, as may be seen from Table II (E–G) with serum samples containing increased total cholesterol content, used as enzymes, there was a marked and proportionate increase in the LCAT activity against heated normal serum samples (about 60% in 3 hrs).

Discussion

Although recent studies (Don P. Jones *et al.*[5], Rutenberg and Soloff[6], and Portman and Sugano[8]) on the serum esterification of cholesterol make use of radioactive cholesterol incorporated into artificially prepared lipoprotein complexes, with a view to obtaining greater sensitivity of the technique in determinations, the present study is confined to the naturally occurring lipoprotein complexes in human serum and cholesterol determinations by the classical colorimetric method using Leiberman Burchard reagent, which in our hands is sensitive for measuring accurately a minimum of 50 μg. of free or esterified cholesterol per sample. Since normal serum cholesterol concentration is of the order of 150 mg%, the technique employed here was well within the bounds of experimental accuracy.

The results of the present study indicate that the normal cholesterol level in young adults of this area vary from 120 mg% to 180 mg% of which 60% is in the ester form and 40% is free alcohol. However, in hypercholesteremia, the increase is predominantly in the ester portion and the free form did not register any appreciable increase in proportion with the absolute increase in total cholesterol.

The esterifying system, with the pre-existing lipoprotein substrate complex in the serum, shows a linear increase with time and in 3 hrs 50% of the free cholesterol is esterified. The enzyme has a pH optimum at 7·4, in agreement with the earlier studies reported (Portman and Sugano[8]).

It is of particular interest as the results in Table II show, that the degree of esterification, reflecting the amount of LCAT activity, is in direct proportion to the total cholesterol status of the blood. The serum having 150% mg of total cholesterol showed 46% esterification, while in a sample with 460 mg% total cholesterol, the esterification was 62%. It is logical, therefore, to hypothesise that hypercholesteremia in blood, which as the data here have indicated to be a reflection of the increased ester compartment, is accompanied by increased LCAT activity in the serum concerned. Both free and esterified cholesterol in the serum are known to be mobilised from the liver in association with specific lipoprotein complexes. Due to the increased serum LCAT activity, the free cholesterol is transferred to the esterified compartment, the liver may be called upon to mobilise more free cholesterol into the blood and since this may be limited by the lipoprotein saturating capacity for free cholesterol, the net increase in hypercholesteremia is in the ester compartment, almost at the expense of the free cholesterol compartment. These results are in agreement with the report of Manger and Nestel[9] who, from a study of 16 subjects, found a similar co-relation between the plasma cholesterol and the rate of its esterification.

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