J. Biosci., Vol. 14, Number 1, March 1989, pp. 55–61. © Printed in India.

Cholesterol binding reserve of hyperlipemic rat serum lipoproteins in chronic ethanol administration

RAMESH CHANDER, CHANAN SINGH and
NARINDER K. KAPOOR*
Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001, India

MS received 26 July 1988; revised 25 January 1989

Abstract. Chronic administration of ethanol in rats caused the reduction of serum cholesterol binding reserve. The very low density and high density lipoproteins, main serum cholesterol binding reserves, were slightly increased with corresponding increases in their lipid and protein components during initial stage of alcohol consumption. However, these capacities get deminished during reversal of hyperlipemia induced by prolonged action of ethanol. This situation may be an early indicator for the initiation of hepatic damage and a variety of secondary effects of ethanol.

Keywords. Serum cholesterol binding reserve; lipoprotein cholesterol binding reserve; hyperlipemic; ethanol administration; liver damage; lipoproteins in alcoholism; lipid metabolism.

Introduction

Hsia *et al.* (1975) have demonstrated that serum can solubilize a considerable amount of exogenously added cholesterol. This capacity of serum was termed as serum cholesterol binding reserve (SCBR) and it accounts for its potential to solubilize cholesterol from arterial walls through reverse cholesterol transport mechanism (Stein *et al.,* 1975). Borresen and Berg (1981a, b) reported that SCBR can be attributed to cholesterol solubilization capacity of two lipoprotein subfractions, one of which contains high density lipoprotein (HDL) as major component, and the other very low density lipoprotein (VLDL). Hence it should and does correlate well with clinical parameters of diseases related with disorders of lipid metabolism. SCBR has been shown to get diminished in myocardial infarction (Chandra *et al.,* 1982), diabetes mellitus (Ghatak *et al.,* 1985) atherosclerosis and hypertension (Chandra *et al.,* 1980) and nephrotic syndrome (Perez *et al.,* 1979). Chronic administration of ethanol has been known to cause marked alterations in lipid and lipoprotein metabolism (Chander *et al.,* 1987, 1988). However information on cholesterol binding reserve of lipoproteins in alcoholism and its relationship with lipoprotein lipids is not available in literature. Therefore, it was considered of interest to study the cholesterol binding reserve of rat serum lipoproteins at various stages of hyperlipemia induced by ethanol feeding and the results are presented in this communication.

CDRI Communication No. 4321.

*To whom all correspondence should be addressed.

Abbreviations used: SCBR, Serum cholesterol binding reserve; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; CBR, cholesterol binding reserve; TC, total cholesterol; PL, phospholipid; TG, triglyceride.

Materials and methods

Male adult rats of Charles foster strain (150–200 g) inbred in CDRI animal house were divided into control and alcohol fed groups of 8 rats in each group. Ethanol was administered in a 50% (v/v) aqueous solution (3·76 g/kg body weight) by gastric tubing once a day for 60 days. Control rats were given normal saline. At the end of the experiment representing 20, 30, 40, 50 or 60 days of alcohol treatment, the animals were fasted overnight, blood was withdrawn by retro orbital plexus and centrifuged. The serum was fractionated into VLDL, LDL and HDL by polyanionic precipitation methods (Burstein *et al.,* 1982) using heparin, dextran sulphate (molecular weight 5×10^5) and $MnCl_2$ as reactants. Each fraction was dialysed, freeze dried and quantitated according to the method of Hervie *et al.* (1973). Cholesterol binding reserve in serum lipoprotein was measured as described earlier by Hsia *et al.* (1975) with slight modifications. Control and hyperlipemic serum (0·5 ml) and lipoproteins (4 mg) dissolved in normal saline were mixed with 7 mg of cholesterol sonicated to a particle size of 10–60 μm and incubated at 37°C in a metabolic shaker with 60 strokes per min for 16 h. After incubation, the undissolved cholesterol was removed by filtering through Whatman No. 42 filter paper and cholesterol estimation in control and experimental sets with or without exogenous cholesterol were carried out by the method of Zlatkis *et al.* (1953). Cholesterol binding reserve was calculated as the difference between the two determinations and expressed in mg per 100 ml, as described by Perez *et al.* (1979). Lipoprotein and serum samples were delipidated with ethanol: ether (3:1 v/v). The apoprotein content was estimated by the method of Radding and Steinberg (1960) and Lowry *et al.* (1951). The lipid extracts were used for the estimation of total cholesterol (TC), phospholipid (PL) (Wagner *et al.,* 1961) and triglyceride (TG) (Handel and Zilversmit, 1957).

Results

Chronic administration of ethanol to rats resulted in increased level of SCBR which was maximum during 30 days of treatment (table 1). However, on further feeding of

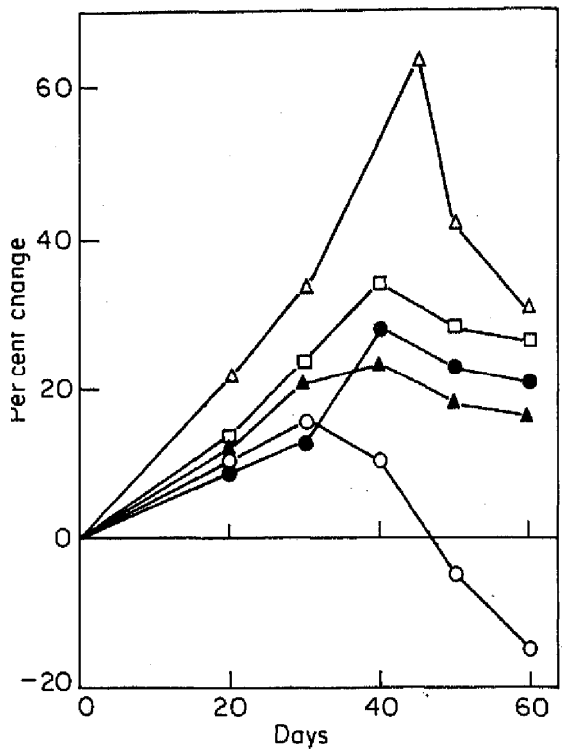
Table 1. Cholesterol binding reserve in hyperlipemic rat serum lipoproteins.

Period of treatment (days)	Experimental schedule	Serum	VLDL	LDL	HDL
20	Control	33·63 ± 2·60	10·98 ± 1·00	0·156 ± 0·010	17·68 ± 1·78
	Ethanol fed	39·52 ± 3·04[a]	13·89 ± 1·44	0·157 ± 0·014[b]	20·14 ± 1·29
30	Control	34·61 ± 2·20	11·81 ± 1·01	0·151 ± 0·010	17·10 ± 1·35
	Ethanol fed	40·74 ± 3·36	14·34 ± 2·07	0·159 ± 0·018[b]	20·76 ± 2·39
40	Control	35·71 ± 2·28	11·62 ± 1·21	0·156 ± 0·020	18·75 ± 1·70
	Ethanol fed	39·01 ± 2·10[a]	13·19 ± 0·68[a]	0·156 ± 0·014[b]	19·49 ± 1·10[b]
50	Control	36·64 ± 2·33	11·70 ± 1·34	0·159 ± 0·014	17·39 ± 1·25
	Ethanol fed	33·47 ± 1·40[a]	10·79 ± 0·68[a]	0·160 ± 0·022[b]	16·38 ± 1·20
60	Control	36·07 ± 2·66	11·75 ± 1·55	0·154 ± 0·020	19·06 ± 1·57
	Ethanol fed	29·94 ± 2·70	9·61 ± 0·90[a]	0·150 ± 0·013[b]	14·25 ± 2·00

Values are expressed as mg/dl ± SD of 6 separate observations.

$P<0·01$. [a] $P<0·05$; [b] P not significant.

ethanol to these animals for 60 days, the levels were observed to decrease by 17% as compared to control. It may be seen that CBR of lipoproteins in control rats did not show any perceptible change during the experimental period of 60 days. The cholesterol binding was maximum in case of HDL, being 1·5-fold more than in VLDL. The levels of CBR of HDL and VLDL as a function of time, were comparable with those of SCBR during the course of treatment and were observed to decrease by 24·40 and 18·20% respectively as compared to control during 60 days of ethanol treatment. LDL of control as well as hyperlipemic rat serum failed to demonstrate the CBR capacity to any significant extent (table 1). It may be seen (figures 1–4) that the levels of serum components *viz.* TC, PL, TG and protein exhibited a progressive increase with marked increase in TG content up to 40 days of ethanol treatment followed by a fall during the subsequent period of treatment. The contents of TC, PL, TG and apoprotein of VLDL, LDL and HDL increased to varying extents, as a result of chronic ethanol intake (figures 2–4). However, the patterns of these alterations as a function of time were similar to those of serum lipids and protein. The VLDL from these animals was found to have 2-fold more TG than the controls. The parameter which shows unique behaviour under the chronic administration of ethanol is the CBR values of serum HDL and VLDL but not of LDL. After attaining its peak value on the 30th day of alcohol treatment it starts declining progressively and becomes much lower than the normal value on the 60th day of treatment.



figures 1. Effect of alcohol feeding on the CBR, lipid and protein contents of hyperlipemic rat serum. (O), SCBR (35·33±1·2); (□), TC (97·3±1·5); (▲) PL (68·75±0·94); (Δ), TG (77·26 ± 2·74);(●), protein (5670±170). Control values (in mg/dl) obtained from 6 rats are given as mean ± SD in parantheses.

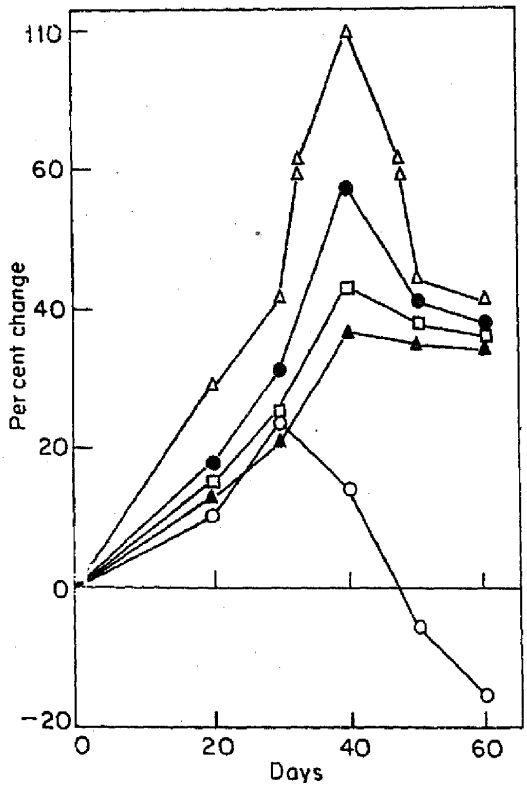


Figure 2. Effect of alcohol feeding on the CBR, lipid and protein contents of rat serum VLDL. (O), CBR (11·27±0·33); (□), TC (4·44±0·06); (▲), PL (5·87±0·28); (Δ), TG (34·12 ±0·85);(●), apoprotein (4·35 ±0·23). Control values (in mg/dl) obtained from 6 rats are given as mean±SD in parantheses.

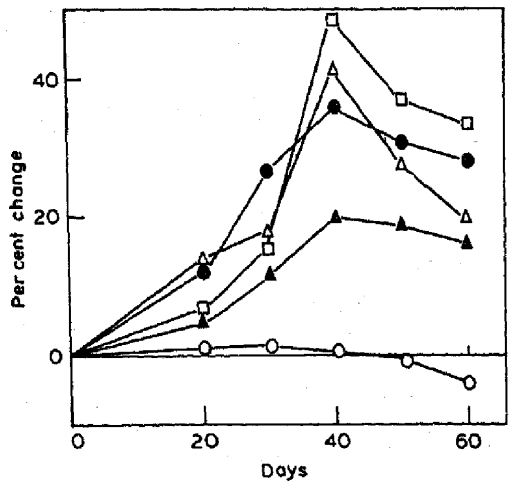


Figure 3. Effect of alcohol feeding on CBR, lipid and protein contents of rat serum LDL. (O), CBR (0·155 ±0·012) (□); TC (8·9±0·32); (▲), PL (7·63±0·17); (Δ), TG(16·72±0·10); (●), apoprotein (14·94±0·24). Control values (in mg/dl) obtained from 6 rats are given as mean ± SD in parantheses.

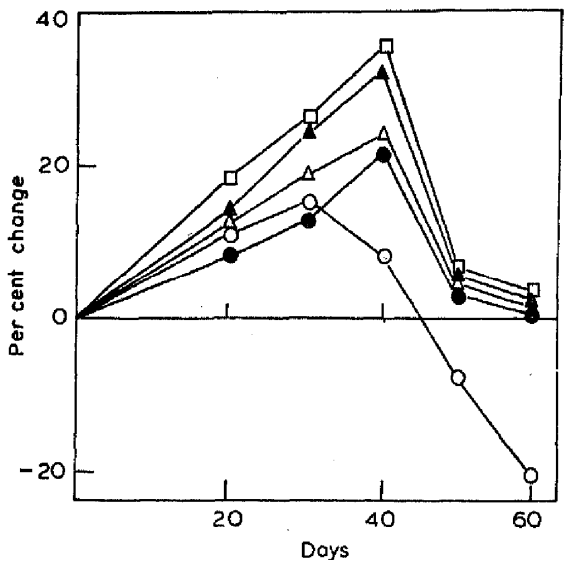


Figure 4. Effect of alcohol feeding on CBR, lipid and protein contents of rat serum HDL. (○), CBR (17·99 ±0·86); (□), TC (38·35 ±0·42); (▲), PL (27·25 ±1·05); (Δ), TG(15·90±0·25); (●), apoprotein (151·16 ±2·72). Control values (in mg/dl) obtained from 6 rats are given as mean±SD in parantheses.

Figures 1–4, show that SCBR and CBR of lipoproteins increase with corresponding increase in their lipids and proteins up to 30 days of treatment. However, these capacities tend to decrease progressively afterwards and do not manifest any relationship with the alterations in the levels of their lipid or protein components under the action of ethanol for 60 days. LDL has least binding capacity in both control as well as in ethanol treated groups, although TC (8·9±0·32mg/dl), PL (7·63±0·17 mg/dl), TG (16·70±0·20mg/dl), and apoprotein (14·94±0·25mg/dl) were increased by about 39, 18, 34 and 36% respectively at peak period of hyperlipemia caused by ethanol treatment.

Discussion

The present study demonstrates the alteration in CBR of serum and its lipoproteins (VLDL and HDL), which in turn are associated with alterations in their lipid and protein components under the action of ethanol. Increased CBR during early treatment with ethanol is likely to enable the serum lipoproteins to remove lipids from tissues of hyperlipemic subject (Kramsch and Hollandu, 1973; Malinow, 1981). The increased production of HDL plays a compensatory role in counteracting fat accumulation with enhanced CBR activity in alcohol fed rats (Aleindor *et al.,* 1970). Increased levels of SCBR and serum HDL cholesterol have been reported in *Curum copticum* (Ajawan) treated rabbits (Agrawala and Pant, 1987). The present findings represent the reversal of hyperlipemia after 40 days along with lowering of lipoprotein lipids and protein levels leading to decreased function of cholesterol binding reserve. The abnormal levels of CBR may indicate the initiation of risk factors manifestable with hyperlipemia followed by transition from fatty liver to more advanced lesions and damage. Borrowsky *et al.* (1976) reported that the

release of liver fat into blood in the initial stage is followed by a relative failure of fat removal from the liver at the advanced stage of hyperlipemia induced by ethanol in baboons which may cause liver damage. Perez *et al.* (1979) revealed that SCBR in normal and patients with nephrotic syndrome tend to increase with increasing serum triglyceride values. However, these levels in normal subjects increase with increase in cholesterol, while the SCBR shows no increase in the nephrotic group. Borresen and Berg (1981a, b) have studied the effect of lipid lowering drug 'Gemsfibrozil' in hyperlipemic humans and found a significant increase in the levels of HDL apoprotein, Apo A_I, Apo A_{II} and SCBR. Miller (1980) has shown that the binding capacities are directly related with surface activity of the intrinsic protein components constituting apoproteins of VLDL and HDL, and any alteration in the levels of these active proteins may influence the CBR of their lipoproteins. The abnormally unique response of CBR after 30 days of ethanol treatment may be due to serious alterations of the protein component (apoprotein) of the lipoproteins. The patients with history of chronic alcoholism have been shown to have structural and functional changes in lipoprotein patterns accompanying hepatic damage (Weidman *et al.,* 1982). CBR of LDL was not affected by ethanol feeding which would suggest that LDL apoprotein fails to contribute towards the binding properties of exogenously added cholesterol. The decline in CBR of lipoproteins (VLDL and HDL) during prolonged alcohol intake may be due to the toxic affects of lipid peroxides and free radicals produced in alcoholism. This is supported by our previous finding on lipid peroxidation of serum lipoproteins in chronic alcohol administration (Chander *et al.,* 1988). The diminished SCBR would be a sensitive marker of hyperbetalipoproteinemia and hepatic damage in alcoholism.

Acknowledgement

One of the authors (C.S.) is grateful to the Council of Scientific and Industrial Research, New Delhi for financial support.

References

Agrawala, J. N. and Pant, M. C. (1987) *Indian J. Med. Res.,* **83,** 93.
Aleinder, L. G., Infanse, R., SolarArgilaga, C, Raisonnier, A., Polonovski, J. and Caroli, J. (1970) *Biochim. Biophys. Acta.,* **210,** 483.
Borowsky, S. A., Perlow, V. W., Baroana, E. and Leiber, C. S. (1976) *Gastroentrology,* **70,** 978.
Borreson, A. L. and Berg, K. (1981a) *Artery.,* **9,** 77.
Borreson, A. L. and Berg, K. (1981b) *Artery.,* **9,** 96.
Burstein, M. and Legmann, P. (1982) *Monogr. Atheroscler.,* **2,** 7882.
Chander, R., Kapoor, N. K. and Singh, C. (1987) *J. Biosci.,* **12,** 55.
Chander, R., Kapoor, N. K. and Singh, C. (1988) *J. Biosci.,* **13,** 269.
Chandra, R., Singh, R., Kumar, S. and Srivastava, D. K. (1980) *Indian Heart J.,* **32,** 355.
Chandra, A., Gupta, S., Bhatia, K. B. and Kapoor, N. K. (1982) in *Proceedings of the world congress on caronary heart disease,* Bombay, February, 1982.
Ghatak, A., Bhatia, K. B., Chandra, A. and Kapoor, N. K. (1985) *Acta Diabetol. Lat.,* **22,** 253.
Handel, E. V. and Zilversmit, D. B. (1957) *J. Lab. Clin. Med.,* **50,** 152.
Harvie, N. R. (1973) *Anal Biochem.,* **53,** 252.
Hsia, S. L, Chao, Y. S., Hennekens, C. H. and Reader, W. B. (1975) *Lancet,* **2,** 1000.
Kramsch, S. M. and Hollandu, W. J. (1973) *J. Clin. Invest.,* **52,** 236.

Lowry, O. H., Rosebrough, N. J., Far., A. L. and Randall, R. J. (1951) *J. Biol. Chem.,***193 ,** 265.
Malinow, M. R. (1981) *Circulation,* **64,** 1.
Miller, G. J. (1980) *Annu. Rev. Med.,* **31,** 97.
Perez, G. O., Levine, S., Gomez, E. and Hsia, S. L. (1979) *Nephron,* **24,** 146.
Radding, C. M. and Steinberg, D. (1960) *J. Clin. Invest.,* **39,** 1560.
Stein, Y., Glanceaud, M. C, Faimaru, M. and Stein, O. (1975) *Biochim. Biophys. Acta,* **380,** 106.
Wagner, H., Horhammer, L. and Wolf, D. (1961) *Biochem. Z.,* **334,** 175.
Weidman, S. W., Ragland, J. B. and Sabesin, S. M. (1982) J. *Lipid Res·,* **23,** 556.
Zlatkis, A., Zake, B. and Royle, A. J. (1953) *J. Lab. Clin. Med.,* **41,** 486.