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Mechanism of hypercholesterolemia produced by biotin deficiency

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Abstract. The effect of biotin deficiency on the metabolism of cholesterol was studied in rats fed cholesterol-free and cholesterol-containing diet. Biotin deficiency induced by feeding raw egg-white resulted in higher cholesterol in the serum and aorta, and higher high density lipoprotein cholesterol and low density lipoprotein + very low density lipoprotein cholesterol. In the liver, cholesterol increased only in the cholesterol diet group but not in the cholesterol-free diet group. Levels of triglycerides were lower in the biotin-deficient, cholesterol-free diet group, but triglycerides were elevated in the cholesterol diet group. Concentration of bile acids in the liver and activity of lipoprotein lipase in the heart and adipose tissue were significantly decreased in the biotin-deficient rats. Release of lipoproteins into the circulation, incorporation of [1,2-^{14}C] acetate into cholesterol, and activity of plasma lecithin: cholesterol acyl transferase were higher.

Keywords. Biotin deficiency; lipoprotein lipase; plasma LCAT.

Introduction

During our investigations on the effect of deficiency of vitamins on cholesterol metabolism, it was found that biotin deficiency produced significant hypercholesterolemia in rats fed cholesterol-free and cholesterol-containing diet. Apart from a report by O'Neill and Bannister (1984) on increased cholesterogenesis in hepatocytes isolated from biotin-deficient chicks and an earlier observation of Scott (1958) on the hypercholesterolemia in a boy having biotin deficiency, no other reports seem to be available in this respect. We have studied the mechanism of this hypercholesterolemic action in biotin deficiency and the results are reported in this paper.

Materials and methods

Female albino rats (Sprague-Dawley strain, weight 60–80 g) were grouped as follows with 15 rats in each group, in two separate experiments (A and B).

A. Cholesterol-free diet:
   1. Biotin-deficient.
   la, Pairfed control group.

B. Cholesterol-containing diet:
   2. Biotin-deficient.
   2a. Pairfed control group.

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Abbreviations used: LCAT, Lecithin cholesterol acyl transferase; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; CoA, coenzyme A.

Biotin deficiency was induced in groups 1 and 2 by feeding raw egg-white. The composition of the basal diet (g/100 g) is given below.

Cholesterol-free diet: Corn starch, 67; casein (vitamin- and fat-free), 5; egg-white, 15; groundnut oil, 8; salt mixture, 4; vitamin mixture (without biotin), 1.

Cholesterol-containing diet: Corn starch, 58; casein, 5; egg-white, 15; coconut oil, 15; salt mixture, 4; vitamin mixture (without biotin), 1; cholesterol, 2.

Wesson's salt mixture was used (Oser, 1965). $ZnCl_2$ and $CoCl_2 \cdot 6H_2O$ were also added to the diet at a concentration of 15 and 0·15 mg/kg diet respectively. Vitamin mixture used contained (per 100 g diet): retinyl palmitate, 1000IU; ergocalciferol, 200 IU; α-tocopherol, 12mg; menadione, 0·3 mg; thiamine hydrochloride, 1mg; riboflavin, 1mg; pyridoxine, 0·6mg; niacin, 10mg; calcium pantothenate, 5mg; inositol, 20mg; choline, 300mg; folic acid, 0·2mg; vitamin B_{12}, 3 μg; *p*-amino-benzoic acid, 5 mg; made up to 1 g with dextrose. No biotin was added.

Rats in groups 1 and 2 received raw egg-white while those of the other groups received the same quantity of boiled egg-white. Since diet consumption in the biotin-deficient groups was lower, pairfed control groups were maintained which received the same amount of biotin-adequate diet. The pairfed control rats received 5 μg of biotin/100 g body weight per day subcutaneously. No biotin was given to the deficient groups. The rats were housed individually in polypropylene cages in rooms maintained at 25 ± 1°C. Water was available *ad libitum.* The duration of the experiment was 75 days. At the end of this period, the rats were deprived of food overnight, stunned by a blow at the back of the neck, and killed by decapitation. Blood and tissues were removed to ice-cold containers for various estimations.

Estimation of biotin in the serum and liver was carried out microbiologically using *Lactobacillus plantarum* as described by Wright and Skeggs (1944) and Baker *et al.* (1962). Total cholesterol and triglycerides in the serum and tissues were determined as described earlier (Menon and Kurup, 1976). For assay of plasma lecithin: cholesterol acyl transferase (LCAT, EC 2·3·1·43), plasma from heparinized blood was immediately extracted with acetone: ethanol (1:1). Another aliquot was incubated at 37°C for 3 h, at the end of which it was extracted with acetone: ethanol (1:1) . Ester cholesterol and unesterified cholesterol were estimated in the lipid extract by the procedure of Schoenheimer and Sperry (1934) and Sperry and Webb (1950). Activity of lipoprotein lipase (EC 3·1·1·3) of the heart and adipose tissue was determined by the method of Krauss *et al.* (1973).

Release of lipoproteins into the circulation was measured using Triton WR 1339 and the estimation of bile acids in the liver was carried out using 3α –hydroxysteroid dehydrogenase as described before (Jaya and Kurup, 1987). Separation of serum lipoproteins into high density lipoprotein (HDL) and very low density lipoprotein (VLDL) + low density lipoprotein (LDL) was carried out as described by Warnick and Albers (1978). *In vivo* incorporation of [1,2-^{14}C]acetate into cholesterol in the liver was carried out as described before (Thomas *et al.,* 1983). Five microcuries of labelled acetate/100 g body weight were administered to the rats.

Protein in the enzyme extract was determined after TCA precipitation by the method of Lowry *et al.* (1951). Statistical analysis was carried out by Student's *'t'* test (Bennet and Franklin, 1967).

Results

Rats fed raw egg-white and given no biotin developed biotin deficiency and exhibited characteristic symptoms like dermatitis, scaly pigmentation and loss of hair. These symptoms were more visible in the cholesterol-free diet group. Avidin present in the raw egg-white forms a stable complex with biotin which is then not absorbed. Rats in the other groups received the same amount of boiled egg-white, in which the avidin is inactivated. This was done to ensure that the protein source was the same in all the groups.

The weight gain was lower in biotin-deficient rats (68 ± 1·7 g) when compared to that of the pairfed controls (75 ± 2·1 g) in the cholesterol-free diet group. The corresponding values for the cholesterol diet group were 80 ± 2·1 and 90 ± 2·3 g respectively. Rats of the deficient groups showed significantly lower biotin levels in the serurn and liver when compared to the corresponding pairfed controls. The values were 0·65 ± 0·016 and 1·10 ± 0·028 (μg/100 ml) for serum and 30·82 ± 0·74 and 55·20 ± 1·55 μg/100 g wet tissue) for liver in the biotin-deficient and pairfed rats respectively for the cholesterol-free diet group. The corresponding values for the cholesterol diet group were 0·83 ± 0·023 and 1·30 ± 0·035 for serum and 42·55 ± 1·10 and 75·30 ± 2·10 for liver.

Concentration of cholesterol and triglycerides

Biotin-deficient rats of the cholesterol-free diet group showed higher cholesterol in serum and aorta when compared to the corresponding pairfed controls. But liver cholesterol was not significantly altered. Our results on liver cholesterol are in agreement with the report of absence of any significant change by Curran (1950) and by Dakshinamurti and Desjardins (1908) in biotin deficiency. In the cholesterol diet group, the cholesterol in serum, liver and aorta showed significant increase when compared to the levels in the corresponding pairfed controls. Biotin deficiency increased both HDL cholesterol and LDL + VLDL cholesterol in both the groups. The inclusion of cholesterol in the diet attenuated the increase in the cholesterol levels in serum in biotin deficiency. Liver cholesterol, which was not significantly altered in the cholesterol-free diet, was increased in the cholesterol diet in biotin deficiency. Triglyceride levels were lower in serum, liver and aorta in the biotin-deficient rats in the cholesterol-free diet group. In the cholesterol diet group, however, biotin-deficient rats showed elevated triglycerides in these tissues (tables 1 and 3).

Incorporation of labelled acetate

Increased incorporation of acetate into hepatic cholesterol (on tissue protein basis) was observed in the biotin-deficient rats when compared to the corresponding pairfed controls. These results are in agreement with the increased cholesterogenesis observed by O'Neill and Bannister (1984) in hepatocytes isolated from biotin-deficient chicks. But biotin-deficient rats showed significantly lower concentration of bile acids in the liver (table 2).

Table 1. Concentration of cholesterol and triglycerides in serum, liver and aorta of biotin-deficient and normal rats fed cholesterol-free and cholesterol-containing diet.

Group	Serum		Liver		Aorta	
	Chl	Tgls*	Chl	Tgls*	Chl	Tgls*
	(mg/100 ml)		(. . . . . ml/100 g wet tissue . . . . .)			
A. Cholesterol-free diet						
1. Biotin-deficient	79·50 ± 2·23[a]	5·23 ± 0·14[a]	405·65 ± 11·35	334·68 ± 8·7[a]	249·60 ± 6·98[a]	598·5 ± 14·96[a]
1a. Pairfed control	64·84 ± 1·75	6·75 ± 0·20	385·45 ± 10·75	395·85 ± 11·5	189·50 ± 5·12	708·3 ± 20·54
B. Cholesterol diet						
2. Biotin-deficient	195·30 ± 5·47[a]	14·75 ± 0·43[a]	1620·65 ± 42·14[a]	1215·5 ± 31·6[a]	426·80 ± 11·95[b]	1768·0 ± 49·5[a]
2a. Pairfed control	151·85 ± 3·80	12·80 ± 0·32	1345·75 ± 32·30	998·8 ± 23·9	385·65 ± 9·25	1505·0 ± 40·6

*Triglycerides are expressed as triglyceride glycerol.
Values are mean (n = 6) ± SEM.
Significance of difference: group 1 vs group 1a; group 2 vs group 2a.
[a] $P < 0.01$; [b] $0.01 < P < 0.05$.
Chl, Cholesterol; Tgls, triglycerides.

Table 2. Incorporation of labelled acetate into hepatic cholesterol, concentration of hepatic bile acids, and activity of lipoprotein lipase in biotin-deficient and normal rats fed cholesterol-free and cholesterol-containing diet.

Group	*In vivo* incorporation of [1,2-^{14}C] acetate into hepatic cholesterol (cpm/mg protein)	Hepatic bile acids (mg/100 g wet tissue)	Lipoprotein lipase (μmol glycerol h/g protein) Adipose tissue	Heart
A. Cholesterol-free diet				
1. Biotin-deficient	9·06 ± 0·24[a]	22·35 ± 0·60[a]	128·55 ± 3·21[a]	25·05 ± 0·65[a]
1a. Pairfed control	6·66 ± 0·18	30·60 ± 0·88	146·02 ± 4·09	31·48 ± 0·92
B. Cholesterol diet				
2. Biotin-deficient	5·25 ± 0·15[a]	35·60 ± 0·89[a]	105·85 ± 2·65[a]	17·95 ± 0·47[a]
2a. Pairfed control	3·79 ± 0·10	43·25 ± 1·17	122·50 ± 3·43	23·84 ± 0·67

Details as in table 1.

Table 3. Concentration of cholesterol in serum lipoprotein fractions and release of lipoproteins into the circulation in biotin-deficient and normal rats fed cholesterol-free and cholesterol-containing diet.

Group	HDL (mg/100 ml serum)	LDL+VLDL (mg/100 ml serum)	Release of lipoproteins into the circulation Concentration of cholesterol (. . . . mg/100 ml serum . . . . .) Saline-injected group	Triton-injected group	Increase in cholesterol (%)
A. Cholesterol-free diet					
1. Biotin-deficient	59·60 ± 1·67[a]	19·75 ± 0·55[a]	80·65 ± 2·09	230·6 ± 6·45	186·0 ± 5·0[a]
1a. Pairfed control	48·53 ± 1·16	14·83 ± 0·38	63·88 ± 1·47	155·23 ± 3·73	143·0 ± 3·4
B. Cholesterol diet					
2. Biotin-deficient	55·70 ± 1·56[a]	137·30 ± 3·98[a]	193·60 ± 5·03	478·25 ± 13·39	147·0 ± 4·3[a]
2a. Pairfed control	41·32 ± 1·07	112·08 ± 2·91	150·95 ± 4·07	341·15 ± 9·21	126·0 ± 3·3

Details as in table 1.

Activity of lipoprotein lipase

The activity of lipoprotein lipase in heart and adipose tissue showed significant decrease in the biotin-deficient rats when compared to the corresponding pairfed controls. But the activity of plasma LCAT was more in the biotin-deficient rats fed cholesterol-free diet. Activity expressed as per cent increase in the ratio of ester cholesterol to free cholesterol during incubation was 35·20 ± 1·03 in the biotin-deficient rats compared to 27·35 ± 0·74 in the pairfed controls (table 2).

Release of lipoproteins into the circulation

There was significantly greater release of lipoproteins into the circulation in the biotin-deficient rats than the corresponding pairfed controls (table 3).

Discussion

The main role of biotin in lipid metabolism is to act as a cofactor for carboxylases, particularly acetyl-coenzyme A (CoA) carboxylase, which catalyses the rate-limiting step in fatty acid synthesis. The decreased concentration of triglycerides in serum and liver in the biotin-deficient rats of the cholesterol-free diet group may be due to the block in the carboxylation of acetyl-CoA to malonyl-CoA. The increase in the serum and liver triglycerides in the biotin-deficient rats of the cholesterol diet group may be due to the high intake of dietary fat. Increased cholesterogenesis, as evidenced by the increased incorporation of labelled acetate into hepatic cholesterol, in the biotin-deficient rats may be due to the fact that more acetyl-CoA (whose utilization for fatty acid synthesis is now decreased) may be diverted for cholesterol synthesis. The fact that liver cholesterol is not significantly different in the cholesterol-free diet group inspite of the increased synthesis of cholesterol in biotin deficiency may be due to the fact that most of the newly synthesized cholesterol is used for lipoprotein synthesis. This is evident from the increased release of lipoproteins into the circulation. This increased release of lipoproteins may also contribute to the hypercholesterolemia and hypertriglyceridemia in biotin deficiency. The increase in aortic cholesterol in the biotin-deficient rats may be due to the increase in the serum LDL + VLDL cholesterol. The major source of cholesterol for the arterial tissue is circulating lipoproteins, particularly LDL.

Hepatic degradation of cholesterol to bile acids is also decreased in the biotin-deficient rats, as indicated by the decreased concentration of bile acids in the liver. This may be due to decreased activity of propionyl-CoA carboxylase, a biotin-dependent enzyme which catalyses the oxidation of propionic acid formed during the conversion of cholesterol to bile acids. The decreased activity of lipoprotein lipase, which is involved in the uptake of circulating triglyceride-rich lipoproteins (chylomicron + VLDL), may result in decreased uptake of these lipoproteins and this, along with the high intake of fat, may contribute to the hypertriglyceridemia in the biotin-deficient rats of the cholesterol diet group. The increase in the activity of LCAT, which is involved in the esterification of free cholesterol in the serum, in the deficient rats may be due to the fact that the substrate for this enzyme, HDL cholesterol, is increased in the deficient rats.

Thus the hypercholesterolemia observed in biotin deficiency may be due to increased synthesis of cholesterol in the liver, decreased breakdown of hepatic cholesterol to bile acids, increased release of lipoproteins into the circulation and their decreased uptake by the extrahepatic tissues.

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