Effect of Bile Duct Cannulation on Plasma Lipoproteins.* (29579)

GREGORY B. CLARKE,† KAICHI FUKAZAWA,‡ FRED A. KUMMEROW AND TOSHIRO NISHIDA

Burnsides Research Laboratory, University of Illinois, Urbana

Bile duct cannulation is known to accelerate both catabolism and biosynthesis of cholesterol in liver(1,2,3). Since liver cholesterol exists in equilibrium with serum cholesterol, a component of plasma lipoproteins, the response of the plasma lipoprotein level to bile duct cannulation may depend upon cannulation-induced changes in the metabolism of cholesterol and of other components of plasma lipoproteins. While studying the metabolism of cholesterol-4-C^{14} in cannulated animals, we have previously noted a marked decrease in the plasma low density lipoprotein levels after cannulation in growing chicks kept on a commercial grain diet(4). The present study revealed that the level of low density lipoproteins and their mixed fatty acid composition in bile duct cannulated chicks are greatly influenced by the type and level of dietary fat.

Methods. Two separate studies were carried out. In the first study, day-old female chicks (New Hampshire-Columbian Cross) were divided into 2 groups of 15 chicks each and kept on experimental diets(5) which contained 35.3% isolated soybean protein and 1% corn oil or hydrogenated coconut oil. In another series 2 groups of 15 chicks each were fed these fats at a 10% level. At 5 weeks of age 8 chicks in each group were selected, the neck of the gall bladder was ligated, and the hepatic and cystic ducts were cannulated with polyethylene tubing(4). Following the operations, the chicks again had access to the experimental diets. Bile was collected at various intervals for a period of 9 days, at which time both the cannulated and the control birds were sacrificed by exsanguination.

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† Present address: Michael Reese Hospital, Chicago, Ill.

‡ On leave of absence, Nagasaki Univ. School of Med., Nagasaki, Japan.

In the second study, the sequence, in one bird per treatment, of lipoprotein changes during bile duct cannulation, ligation, and in the absence of treatment was followed in adult male birds which had been fed for 2 years a commercial grain diet containing approximately 4% fat. A blood sample was removed from the wing vein 4 days before the operation and at 3- to 6-day intervals following the operation. On the 35th day the birds were sacrificed by exsanguination.

Isolation and analysis of the serum lipoproteins of each animal were carried out according to the ultracentrifugal flotation method described by DeLalla and Gofman (6). The fatty acid composition of the lipid moiety of the lipoproteins was determined on pooled samples extracted with a 2:1 chloroform-methanol mixture(7). The methyl esters were prepared by trans-esterification(8) and analyzed by gas liquid chromatography on a model 90C Aerograph with a 10-foot diethylene glycol succinate column at 215-220°C and with a helium flow rate of 50 ml per minute(9).

The bile samples were pooled and sodium hydroxide was added so as to make the solution approximately 5 N. The resulting mixture was then saponified for 3 hours at 120°C under 20 lb pressure. After saponification, an equal volume of 95% ethyl alcohol was added to the hydrolysate and the unsaponifiable materials were extracted with petroleum ether. The aqueous phase was acidified with hydrochloric acid and the fatty acids extracted with petroleum ether. The methyl esters of these fatty acids were then prepared and their composition was determined by gas-liquid chromatography.

Results. The changes in the low density lipoprotein level of chicks after bile duct cannulation were greatly influenced by the type and level of dietary fat (Table I). A decrease in level of the serum low density

TABLE I. Effect of Dietary Fat on Changes in Concentration of Low Density Lipoproteins of Chicks 9 Days After Bile Duct Cannulation.

	Dietary supplements			
	Exp 1		Exp 2	
	1% Corn oil	1% Hydrogenated coconut oil	10% Corn oil	10% Hydrogenated coconut oil
% Change in concentration of low density lipoproteins in individual cannulated chicks*	—56%	—68%	+232%	+100%
	—45	—67	+200	+ 85
	—42	—67	+ 93	+ 12
	—41	—51	+ 26	+ 10
	—33	—31	+ 13	— 7
	—23	—22		
	—20	—14		
Low density lipoprotein concentration in non-cannulated chicks (mg/100 ml)†	111.5	112.3	79.5	85.2

* The signs + or — indicate increase or decrease, respectively, as compared to concentration in non-cannulated chicks. Eight chicks in each group were cannulated; values tabulated are for successfully performed cannulations only. Exp 1 and 2 were conducted separately, therefore changes in concentration of low density lipoproteins were expressed as % rather than as absolute values so that changes in all 4 groups could best be compared.

† Values represent low density lipoprotein concentration of pooled sera from 7 non-cannulated chicks in each group.

lipoproteins, as compared to the level found in corresponding non-cannulated chicks, occurred after bile duct cannulation in chicks which had received 1% dietary fat. When the chicks received a diet containing 10% fat, an increase rather than a decrease occurred in the low density lipoproteins of birds cannulated for the same period as the birds in the low fat groups. Moreover, the nature as well as the level of the dietary fat affected the serum low density lipoprotein level. The cannulated birds fed 10% corn oil showed a greater increase in lipoprotein concentration than did birds which had received 10% hydrogenated coconut oil.

To observe the sequence of serum low density lipoprotein changes caused by bile duct cannulation and ligation, adult male birds were used, thus allowing periodic removal of blood samples sufficient for ultracentrifugal analysis of plasma low density lipoproteins. The bird which had been kept on a commercial grain diet showed an elevated lipoprotein level 7 days after cannulation and maintained this elevated level for the next 8 days, after which there was a steady decline in lipoprotein concentration (Table II). Elevation of lipoprotein level in the cannulated bird was much less pronounced than in the ligated bird. Repetition of the experiment produced data consistent with these findings. In spite of the susceptibility of the low density lipoproteins to change following cannulation, the high density lipoprotein levels in both growing and adult male birds showed little change from the initial concentration.

Elevation of low density lipoproteins noted in the chicks fed a high fat diet and in adult birds fed the commercial diet was mostly

TABLE II. Changes in Low Density Lipoprotein Levels (mg/100 ml Serum) in Adult Male Birds After Bile Duct Cannulation or Ligation.*

	Days after operation								
Treatment	0†	3	7	11	15	20	25	31	35
Cannulation‡	36	33	128	122	117	99	80	56	51
Ligation‡	36	133	446	644	1212	758	480	—	572
None	30	35	37	33	—	54	—	23	22

* For each treatment listed, figures represent the sequence of lipoprotein changes in one adult male bird.

† Values at 0 day were obtained for samples taken 4 days prior to operation.

‡ Operation was performed on both hepatic and cystic ducts.

TABLE III. Fatty Acid Composition of Lipid Moiety in Low and High Density Lipoproteins.*

Fatty acid component	Dietary supplements: 10% Corn oil				1% Corn oil				10% Hydrogenated coconut oil				1% Hydrogenated coconut oil			
	Non-cannulated		Cannulated		Non-cannulated		Cannulated		Non-cannulated		Cannulated		Non-cannulated		Cannulated	
	L.D.†	H.D.	L.D.	H.D.	L.D.	H.D.	L.D.	H.D.	L.D.	H.D.	L.D.	H.D.	L.D.	H.D.	L.D.	H.D.
	%															
Caprylic	.2	—	.3	—	—	—	—	—	.2	—	.3	—	—	—	—	—
Capric	.3	—	.3	—	—	—	—	—	.3	—	.3	.1	—	—	—	—
Lauric	1.7	—	.4	.2	—	—	—	—	6.4	2.1	3.7	.8	—	—	—	—
Myristic	1.7	.7	.5	.6	—	.3	.7	.7	6.9	4.5	5.7	2.2	.8	1.2	1.8	1.5
Palmitic	24.5	24.5	23.5	25.3	24.7	23.0	35.2	26.1	27.5	23.9	24.4	22.5	23.6	27.3	39.0	23.0
Palmitoleic	2.0	1.0	1.9	2.4	5.8	4.9	5.0	6.8	6.6	6.0	7.4	7.6	10.0	8.0	3.8	9.6
Stearic	17.1	26.5	18.7	23.6	19.0	13.1	26.9	10.0	12.2	21.2	11.6	17.7	17.6	13.9	29.6	13.6
Oleic	19.3	15.0	20.5	16.3	34.2	40.3	25.5	42.3	26.4	26.2	39.2	35.2	37.2	38.5	24.7	44.3
Linoleic	33.2	32.2	34.0	31.7	17.3	18.3	6.6	14.1	13.4	16.1	7.5	14.0	10.8	11.2	1.0	8.1

* Values represent fatty acid composition of pooled lipoprotein samples. Each non-cannulated group consisted of 7 chicks. Other figures are data from successfully cannulated birds, as explained in Table I.

† L.D. and H.D. indicate low and high density lipoproteins, respectively.

limited to the low S_f range, rather than the range above S_f 20, where the main increases in low density lipoproteins due to hyperlipemia are observed. In the chicks fed a high fat diet, as in adult birds, we observed that elevation was followed by a stationary period and by a decline in lipoprotein concentration.

The fatty acid composition of the lipid portion of lipoproteins from the cannulated chicks which had received 10% corn oil was the same as that found in the corresponding non-cannulated group (Table III). However, the chicks which had received 10% hydrogenated coconut oil did not maintain normal fatty acid patterns in the lipoproteins after bile duct cannulation; a decrease in linoleic acid content was accompanied by a subsequent rise in oleic acid. The linoleic acid content and, to a lesser extent, the oleic acid content, in the low density lipoproteins decreased after bile duct cannulation in the 2 groups of chicks which had received 1% corn or hydrogenated coconut oil, the decrease being more pronounced in the latter group. Accompanying the decreases in both groups was a rise in level of the saturated fatty acids in the lipoproteins. Fatty acid constituents with carbon number above 18 were not determined in the present study. However, due to the relatively small amounts of C_{20} trienoic and C_{20} tetraenoic acids we noted previously in plasma lipoproteins of chicks(10), it is possible that only minor changes had occurred in these fatty acids.

The changes in linoleic acid content in the lipid moiety of the high density lipoproteins closely paralleled those in the low density lipoproteins, but were less marked. This may be partially attributed to the greater stability of the high density lipoprotein concentration after cannulation as compared to the relatively instable low density lipoprotein concentration. The fatty acids isolated from the bile of cannulated chicks (Table IV) showed a close similarity in composition to the fatty acids isolated from the serum lipoproteins of non-cannulated chicks.

Discussion. The effect of dietary fat levels on plasma lipoprotein levels after bile duct

TABLE IV. Fatty Acid Composition of Bile Lipids.*

	Dietary supplements			
	Corn oil		Hydrogenated coconut oil	
	10%	1%	10%	1%
	%			
Lauric	.4	—	.4	.1
Myristic	.4	.4	2.1	.6
Palmitic	33.3	30.0	30.1	28.4
Palmitoleic	2.4	5.8	6.8	7.5
Stearic	24.4	19.3	18.3	16.3
Oleic	10.9	29.3	28.1	37.4
Linoleic	28.3	15.4	14.3	9.4

* Values are for pooled bile samples from successfully cannulated chicks, as explained in Table I.

cannulation may reflect the availability of lipids which were utilizable for biosynthesis of serum lipoproteins. It has been widely recognized that bile duct cannulation, which interrupts the enterohepatic circulation of bile acids, accelerates the turnover of cholesterol. Since cholesterol exists in circulation as a component of plasma lipoproteins, the extent of incorporation of newly synthesized or mobilized cholesterol into plasma lipoproteins may partly be governed by the availability of fatty acids in the cholesterol esters and in other lipid components of the lipoproteins.

The rapid decrease in plasma low density lipoproteins after bile duct cannulation in chicks on low fat diets may be a result of the following factors: 1) decreased availability of dietary fatty acids for lipoprotein synthesis, which was possibly made more pronounced by poor absorption of dietary fat due to the absence of bile salts in cannulated chicks, 2) limited availability of fatty acids from small fat reserves, characteristic of growing chicks, especially of those on low fat diets, and 3) endogenous sources of cholesterol and other lipids which were insufficient to compensate for increased plasma lipoprotein catabolism induced by accelerated cholesterol catabolism and by the loss of fatty acids as bile lipid components which are partially reabsorbed in non-cannulated chicks. Bile duct cannulation in dogs results in an absorption of approximately 40% of the dietary fats as compared to that of the intact animal(11,12). It is not known, however, to what extent loss of bile from cannulated chicks quantitatively interfered with the absorption of dietary fats.

We have noted a greater excretion of bile acids by cannulated chicks fed 10% corn oil than by cannulated chicks on other diets, which is in agreement with the observation of Lewis(13). In spite of such enhanced catabolism of cholesterol, the lipoprotein levels of the chicks kept on 10% corn oil remained at a more elevated level. This phenomenon was probably initiated by an excessive biosynthesis of cholesterol or an increased mobilization of tissue cholesterol. Similarity in fatty acid composition of the low density lipoproteins from both cannulated and non-cannulated chicks which had received 10% corn oil may indicate that even after cannulation this group absorbed and mobilized fat in quantities sufficient to elevate plasma lipoprotein levels for a prolonged period.

In the present study adult birds kept on the commercial diet which had contained 4% fat exhibited elevated plasma lipoprotein levels after bile duct cannulation, in contrast to the previous results obtained in growing chicks kept on the same commercial diet(4). This difference between adult and growing chicks may be due to the fact that the adult birds had well developed adipose tissue which served as an available source of lipids for rapid mobilization, while the growing chicks had very little fat reserve due to the necessity of using a large portion of the dietary fat for growth. Thus the adult birds could sustain elevated lipoprotein levels for a longer period than the growing chicks. It is possible that in the adult birds the action of sex hormones might also have been involved in the prolonged elevation of plasma lipoprotein levels by mechanisms unknown.

A decrease in linoleic acid content of the lipid moiety of the lipoproteins after bile duct cannulation in chicks fed hydrogenated coconut oil or 1% corn oil seemed to be caused by the loss of this essential fatty acid, together with other fatty acids, as a bile lipid component. In these cases the linoleic acid intake was either nonexistent or too inade-

quate to supply the necessary amount of essential fatty acid. The fatty acid patterns of the bile lipid fractions have recently been reported to reflect those of the dietary lipids (14). Our study, however, indicated that they more closely resembled the fatty acid patterns of plasma lipoproteins.

Summary. The effect of bile duct cannulation on the plasma lipoproteins was markedly influenced by the type and level of dietary fat and by the age of the birds. The low density lipoprotein level of cannulated chicks fed 1% fat decreased, while the level in cannulated chicks fed 10% fat increased; the greater increase occurred in the group fed corn oil. High density lipoprotein levels were unaffected. The increases in level of low density lipoproteins in the chicks fed 10% fat were followed by a stationary period and by a gradual decline as observed in cannulated adult birds.

The fatty acid composition of the lipoproteins also changed after cannulation. In all cannulated birds except those fed 10% corn oil the linoleic acid content of the low density lipoproteins decreased. Fatty acid composition of the high density lipoproteins showed closely parallel changes. The mixed fatty acid composition of the bile from cannulated chicks and that from corresponding serum lipoproteins of non-cannulated chicks were very similar.

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Growth Stimulating Effect of 4-Methyl-5(2-Hydroxyethyl) Thiazole on Thiamine-Deficient Rats.* (29580)

F. W. LEAVER AND B. R. WOODY (Introduced by S. Katsh)

Research Laboratories, V. A. Hospital, and Department of Pharmacology, University of Colorado School of Medicine, Denver

It is well known that 4-methyl-5(2-hydroxyethyl) thiazole (MHET) can replace thiamine as a nutrient for some bacteria(1). Abderhalden(2,3) without success attempted to demonstrate that MHET could replace thiamine in the diet of the rat. Since then, MHET and the pyrimidine moiety of thiamine (2-methyl-4-amino-5-hydroxymethyl pyrimidine) have been isolated from urine of rats after giving large doses of thiamine(4,5), suggesting that the rat is capable of cleaving thiamine to give these two products. Also Van Eys(6) has identified MHET as a component of glycerol phosphate dehydrogenase, which indicates a strong possibility that MHET is biologically active. It, therefore, seemed desirable to investigate once again the possibility that MHET is biologically active. As workers in the past have apparently achieved essentially negative results(2,3,6,8)

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