THE ROLE OF BILE ACIDS ON THE PLASMA LIPIDS IN CHICKS GIVEN DIETS CONTAINING MEDIUM CHAIN TRIACYLGLYCEROL

R. T. Mabayo, M. Furuse[1], C-P, Yang[2] and J. Okumura

Laboratory of Animal Nutrition, School of Agriculture
Nagoya University, Nagoya 464-01, Japan

Summary

The effects of the prevention of micelle formation and bile acid reabsorption, by using cholestyramine (CHOLN), a bile acid binding polymer, on the plasma lipid of Single Comb White Leghorn male chicks given diets containing medium chain triacylglycerol (MCT) and long chain triacylglycerol (LCT) were investigated. Corn oil and glyceryl tricaprylate were used as LCT and MCT sources, respectively. Plasma HDL cholesterol was reduced by CHOLN in all treatments. Plasma LDL cholesterol was reduced by CHOLN in chicks given LCT diet but not in MCT diet which could be accounted to the reduced plasma total cholesterol in LCT diet with CHOLN. It is concluded that bile acid binding does not alter the cholesteremic effect of MCT in the plasma of chicks.
(**Key Words**: Chick, Bile Acids, Cholestyramine, Medium Chain Triacylglycerol)

Introduction

Medium Chain Triacylglycerols (MCT) are absorbed and metabolized differently from long chain triacylglycerols (LCT) in mammals. According to Greenberger et al. (1966), MCT can be hydrolyzed to medium chain fatty acids (MCFA) and glycerol in the lumen at low concentration of pancreatic enzymes. The path for the transport of MCFA is both through the portal vein and lymph, but the corresponding long chain fatty acids (LCFA) are transported entirely in the lymph (Bloom et al., 1951). LCFA form mixed micelles with bile acids, although MCFA are well absorbed in the absence of bile acids (Garret and Young, 1975).

MCT has been reported to increase blood cholesterol concentration in human (Hill et al., 1990) and in chickens (Fisher and Kaunitz, 1964). On the other hand, cholestyramine (CHOLN) is basic anion exchange resin which sequesteres bile acids in the intestine and prevent their reabsorption and their enterohepatic circulation. If CHOLN is administered the result is a decreased absorption of exogenous cholesterol (see Packard and Shepherd, 1982) and an increase in bile acid synthesis from endogenous cholesterol in the liver. Consequently, it is believed to lead to an increase in the LDL receptors on liver cells which helps to increase removal of LDL from the blood (Shepherd et al., 1980). Since CHOLN was reported to lower plasma cholesterol in cholesterol-fed cockerels (Tennent et al., 1960), CHOLN may suppress the cholesteremic effect of MCT in the chicken's blood.

This study therefore aims to investigate whether the plasma lipids of chickens given diets containing MCT are altered by preventing the micelle formation and reabsorption of bile acids.

Materials and Methods

One-day old Single Comb White Leghorn male chicks were given a commercial chick mash (CP 21.5%, ME 12.1 kJ/g, Marubeni Shiryo Ltd., Tokyo, Japan) for 7 days. On day 8, the chicks were individually weighed after starving overnight and were selected and distributed into 6 groups of 5 chicks each, so that mean body weights were as uniform as possible. The birds were individually housed in stainless-steel metabolism cages and were fed with experimental diets from day 8 to 17.

The experiment was under equalized feeding conditions. The composition of CHOLN-free diets is shown in table 1. Corn oil and glyceryl tricaprylate were used as LCT and MCT sources, respectively. Questran® as the source of CHOLN was

[1]Address reprint requests to Dr. M. Furuse, Laboratory of Animal Nutrition, School of Agriculture, Nagoya University, Nagoya 464-01, Japan.

[2]Laboratory of Animal Nutrition, National Taiwan University, Taiwan.

Received January 18, 1994
Accepted June 2, 1994

added at 2% level at the expense of corn starch. Dietary MCT or LCT source was at 20% level and each diet was given at 100 g/bird/10 days. The ME, however, were 15.9 and 17.2 kJ/g for MCT and LCT diets, respectively, being lower in MCT diet, so an adjusted LCT diet was included in the study in which the ME was 17.3 kJ/g and was given at 91.9 g/bird/10 days. The composition of the adjusted LCT diet was recalculated in order to provide the amount of daily nutrients equal to the groups given MCT diet. The amount of corn starch was reduced to adjust the amount of protein, vitamins and minerals. Feeding schedule is shown in table 2. The birds in MCT diet and adjusted LCT diet had equal daily ME intake.

On day 18, the chicks were killed by decapitation and blood sampling was done to analyze plasma triacylglycerols and cholesterol. Plasma total cholesterol and HDL cholesterols were determined by a kit (Boehringer Manheim Standards, Manheim, Germany) and total triacylglycerols were determined by a kit (Triglyceride Test®, Wako Pure Chemical Industries, Osaka, Japan). LDL cholesterol was calculated by the method of Friedewald et al. (1972).

One way analysis of variance was applied to the data and Duncan's multiple range test was used to assess the significance between means. All of the data analysis were done using a commercial statistical package (SAS, 1985).

TABLE 1. COMPOSITION OF CHOLESTYRAMINE FREE DIETS (g / kg)

Ingredient	MCT	LCT
Isolated soybean protein	226	226
Mineral mixture[1]	58.8	58.8
Vitamin mixture[1]	2	2
Choline chloride	1.5	1.5
L-Methionine	2.9	2.9
Inositol	1	1
Glycine	4.2	4.2
L-Threonine	1.2	1.2
Cellulose	100	100
Corn starch	402.4	402.4
Corn oil	20	200
Coconado RK®[2]	180	0

[1] Furuse & Okumura (1989).

[2] Coconado RK®, glyceryl tricaprylate (donated by Kao Corp., Wakayama, Japan).

TABLE 2. DAILY FOOD INTAKE OF CHICKS GIVEN DIETS CONTAINING 20% MEDIUM (MCT), LONG CHAIN TRIACYLGLGYCEROL (LCT) OR ADJUSTED LCT DIETS WITH (2%) OR WITHOUT (0) CHOLESTYRAMINE FOR 10 DAYS

Day	Food intake (g)	
	MCT or LCT	Adjusted LCT
1	5	4.60
2	6	5.51
3	7	6.43
4	8	7.35
5	9	8.27
6	11	10.11
7	12	11.03
8	13	11.95
9	14	12.87
10	15	13.79
Total	100	91.90

Results

The plasma lipids of chicks fed diets containing MCT or LCT with or without CHOLN is shown in table 3. In chicks given MCT diet, the plasma total cholesterol was not affected by the addition of CHOLN, but was significantly decreased in chicks given LCT diet. Plasma HDL cholesterol in both triacylglycerol sources was decreased significantly by CHOLN. When CHOLN was added in the diets, plasma HDL cholesterol in chicks fed MCT diet was significantly higher than in the LCT groups. Between groups given CHOLN-free diets, the chicks given adjusted LCT diet had significantly lower plasma HDL cholesterol than those given MCT diet. Plasma LDL cholesterol significantly decreased in chicks given LCT diet when CHOLN was added, but significantly increased in chicks given MCT diet. The addition of CHOLN in adjusted LCT group slightly lowered plasma LDL cholesterol. Between CHOLN-free diets, the adjusted LCT group had significantly lower LDL cholesterol than the other LCT group. The chicks given MCT diet containing CHOLN had the highest plasma LDL cholesterol than all other groups. Plasma total triacylglycerol was not affected by either triacylglycerol sources or CHOLN.

TABLE 3. PLASMA LIPIDS OF CHICKS FED DIETS CONTAINING MEDIUM (MCT) OR LONG CHAIN TRACYLGLYCEROL (LCT) WITH (2%) OR WITHOUT (0) CHOLESTYRAMINE UNDER EQUALIZED FEEDING[2] CONDITIONS

Parameter	Triacylglycerol source						Residual mean square
	MCT		LCT		Adj. LCT[1]		
	Cholestyramine level (%)						
	0	2	0	2	0	2	
Total cholesterol	225[a]	248[a,*]	223[a]	103[c]	178[b]	114[c]	456
HDL cholesterol	116[a]	71[c,*]	104[ab]	43[d]	91[b]	49[d]	223
LDL cholesterol	96[bc]	163[a,*]	109[b]	45[d]	72[cd]	51[d]	504
Total triacylglycerol	60	70[*]	54	72	70	69	49.2

[1] Details are given in Materials and Methods.
Values are in mg/100 ml plasma.
Values are means of 5 birds.
[*] One missing value.
[a-d] Means in a row not having the same superscript letters are significantly different at $p < 0.05$.

Discussion

This study was done to evaluate the effects of preventing the micelle formation and enterohepatic circulation of bile acids, by using CHOLN, on the plasma lipids of chicks given dietary MCT. CHOLN is an anion exchange polymer which irreversibly binds intraluminal bile acids as well as other components of normal luminal micelles, including phospholipid, monoacylglycerol, fatty acid and cholesterol (Vahouny et al., 1980). Bile acid excreted in chicks given MCT diet with CHOLN was lower than in those given diets containing LCT (Mabayo et al., unpublished data).

It was reported that MCT reduced plasma cholesterol in calves (Stewart et al., 1978) and in rats (Fisher and Kaunitz, 1964; Leveille et al., 1967) but increased plasma cholesterol in chicks (Fisher and Kaunitz, 1964; Whiteside et al., 1965). In this study, however, no difference in total plasma cholesterol concentration was observed between groups fed MCT and LCT diets, but was lower in adjusted LCT group, which may mean that at the same ME intakes, LCT would be more hypocholesteremic than MCT in chicks. Polyunsaturated fatty acids (which constitute more than 50% in corn oil) are reported to reduce plasma cholesterol in humans (Hill et al., 1990) which might be true to chicks. In laying hens, serum cholesterol level in the polyunsaturated fatty acid supplemented group was lower than in the control group (Furuse et al., 1992). The addition of CHOLN in the LCT diet reduced plasma cholesterol in chicks, but did not alter in those given MCT diet. Bile acid excretion in chicks given MCT diet was lower than those given LCT diet when CHOLN was added (Mabayo et al., unpublished data) which may mean that the loss of bile acid in chicks given MCT diet was neither effective enough to reduce cholesterol synthesis nor have enhanced bile acid synthesis from endogenous cholesterol.

The major lipoprotein involved in the return of cholesterol from the peripheral cells to the liver is HDL. Plasma HDL cholesterol concentrations were reduced by CHOLN in all treatments which could have been directly related to the excretion of bile acids. The need for cholesterol to synthesize bile acids arises when bile acids are excreted so the liver uptake of HDL could have been enhanced since cholesterol from HDL is the major precursor of bile acids (Fielding and Fielding, 1985). Pieters et al. (1993) have shown that cholesterol esters delivered by apolipoprotein LpA-1 are efficiently coupled to bile acid synthesis. This is being shown by the slight decrease of HDL cholesterol in MCT-CHOLN fed chicks and drastic decrease of HDL cholesterol in LCT-CHOLN fed group which should have been a result from the slight increase of bile acids excreted in MCT-CHOLN fed chicks and the doubled excretion of bile acids in LCT-CHOLN fed chicks as mentioned above.

VLDL and its product, LDL are the major lipoproteins involved in the delivery of cholesterol

from the liver to the peripheral tissues. It was observed that caprylic acid did not suppress LDL-receptor activity, hence, it did not increase plasma LDL concentration in rats (Woollet et al., 1992). In this study, no difference was found in plasma LDL cholesterol concentrations between MCT and LCT groups without CHOLN. However, LDL concentrations in LCT feeding were efficiently decreased by CHOLN treatment. The liver does 90% of LDL removal from the plasma by the receptor-dependent pathway (Meddings and Dietschy, 1987) and CHOLN was shown to increase LDL-receptor synthesis and activity (Shepherd et al., 1980) which may explain the decrease of LDL cholesterol concentration in the chicks given LCT diet. It is likely that the reduced total plasma cholesterol also have reduced the LDL cholesterol concentration. The addition of CHOLN to MCT diet, however, have conversely increased the plasma LDL concentration in chicks in which the increase could not be explained.

From this study, it is concluded that bile acid binding does not alter the cholesteremic effect of MCT in the plasma of chicks. The action of CHOLN may be altered by dietary triacylglycerol sources.

Literature Cited

Bloom, B. L., L. L. Chaikoff and W. O. Reinhardt. 1951. Intestinal lymph as pathway for transport of absorbed fatty acids of different chain lengths. Am. J. Physiol. 166:451-455.

Fielding, C. J. and P. E. Fielding. 1985. Cholesterol Metabolism In: Biochemistry of Lipids and Membranes. edited by D. E. Vance, and J. E. Vance. California: Benjamin/Cummings, p. 404-474.

Fisher, H. and H. Kaunitz. 1964. Effects of medium and long chain saturated triglycerides on blood and liver cholesterol of chickens and rats. Proc. Soc. Exp. Biol. Med. 116:278-280.

Friedewald, W. T., R. I. Levy and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preperative ultracentrifuge. Clin. Chem. 18:499-502.

Furuse, M., R. Okada, K. Kita, K. Asakura and J. Okumura. 1992. Effect of gamma-linolenic acid on lipid metabolism in laying hens. Comp. Biochem. Physiol. 101A:167-169.

Furuse, M. and J. Okumura. 1989. Effect of dietary acetic acid levels on protein and energy utilization in chicks. Poult. Sci. 68:795-798.

Garret, R. L. and J. R. Young. 1975. Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. J. Nutr. 105:827-838.

Greenberger, N. J., J. B. Rodgers and K. J. Iseelbacher. 1966. Absorption of medium and long chain triglycerides: factors influencing their hydrolysis and transport. J. Clin. Invest. 45:217-227.

Hill, J. O., J. C. Peters, L. L. Swift, D. Yang, T. Sharp, N. Abumrad and H. Greene. 1990. Changes in blood lipids during six days of overfeeding with medium or long chain triglycerides. J. Lipid Res. 31:407-416.

Leveille, G. A., R. S. Pardini and J. A. Tillotson. 1967. Influence of medium-chain triglycerides on lipid metabolism in the rat. Lipids 2:287-294.

Meddings, J. B. and J. M. Dietschy. 1987. Regulation of plasma low density lipoprotein levels: New strategies for drug design. Prog. Clin. Biochem. Med. 5:3-23.

Packard, C. J. and J. Shepherd. 1982. Review: The hepatobiliary axis and lipoprotein metabolism: effects of bile acid sequestrants and ileal bypass surgery. J. Lipid Res. 23:1081-1098.

Pieters, M. N., G. R. Castro, D. Schouten, P. Duchateau, J-C. Fruchart and T. J. C. Van Berkel. 1993. Cholesterol esters selectively delivered *in vivo* by high-density-lipoprotein subclass LpA-I to rat liver are processed faster into bile acids than are LpA-I/A-II-derived cholesterol esters. Biochem. J. 292:819-823.

SAS Institute Inc.: SAS User's Guide: Statistics. 1985. SAS Institute, Inc., Cary, NC.

Shepherd, J., C. J. Packard, S. Bicker, T. D. Veitch Lawrie and G. Morgan. 1980. Cholestyramine promotes receptor-mediated low-density-lipoprotein catabolism. N. Engl. J. Med. 302:1219-1222.

Stewart, J. W., K. D. Wiggers, N. L. Jacobson and J. Berger. 1978. Effect of various triglycerides on blood and tissue cholesterol of calves. J. Nutr. 108:561-566.

Tennent, D. M., S. Henry, M. F. Zaretti, G. W. Kuron, W. H. Ott and F. J. Wolf. 1960. Plasma cholesterol lowering action of bile acid binding polymers in experimental animals. J. Lipid. Res. 6:469-473.

Vahouny, G. V., R. Tombes, M. M. Cassidy, D. Kritchevsky and L. Gallo. 1980. Dietary fibers: V. Binding of bile salts, phospholipids and cholesterol form mixed micelles by bile acid sequestrants and dietary fibers. Lipids 15:1018-1021.

Whiteside, C. H., H. B. Fluckiger and H. P. Sarett. 1965. Plasma and liver cholesterol levels in chicks fed medium chain triglycerides (MCT) and cholestyramine. Proc. Soc. Expt. Biol. Med. 118:77-79.

Woollett, L., D. K. Spady and J. M. Dietschy. 1992. Regulatory effects of the saturated fatty acids 6:0 through 18:0 on hepatic low density lipoprotein receptor activity in the hamster. J. Clin. Invest. 89:1133-1141.