THE EFFECT OF DIETARY FATS ON THE HEPATIC AND INTESTINAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITIES IN CHICKS

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Summary

This experiment was designed to evaluate the effect of degree of unsaturation (Experiment 1) and the chain length of constituent fatty acids of dietary fats (Experiment 2) on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activities in the liver and small intestine of chicks.

Chicks were fed experimental diets for 10 days and then killed for the determination of the HMG-CoA reductase activities in the intestinal epithelial cell and hepatic microsomes. The hepatic HMG-CoA reductase activity showed the highest value in chicks fed the tallow-containing diet. Chicks fed diets containing safflower or coconut oil resulted in a significantly lower intestinal HMG-CoA reductase activity in comparison with those fed the olive oil-containing diet.

The hepatic HMG-CoA reductase activity was significantly higher when fat-free and trilaurin were fed than when any other triglycerides were fed. This activity showed the lowest value in the chicks fed the diet containing tristearin. The HMG-CoA reductase activities in the jejunum and ileum were significantly or tended to be higher when trilaurin was fed than when any other triglycerides were fed. Except when trilaurin was fed, the presence of saturated fat in the diet did not have a significant effect on the intestinal HMG-CoA reductase activity, unlike the effect shown when a highly unsaturated fat was added to the diet. There was no significant correlation between the HMG-CoA reductase activities of the liver and intestinal, and the HMG-CoA reductase activity and cholesterol content of the intestinal epithelial cells.

(Key Words: HMG-CoA Reductase, Intestine, Liver, Dietary Fat, Chicks)

Introduction

Intestinal mucosa has been demonstrated to be second only to the liver as a source of endogenously synthesized cholesterol in the rat (Dietschy and Siperstein 1967, Dietschy and Wilson 1968). In the rat, for example, sterol synthesis of the intestinal mucosa accounts for about 24% of whole-body sterol synthesis, compared to 50% for the liver (Tarley et al., 1981). It has been suggested recently, however, that even under conditions of low dietary cholesterol intake, cholesterol synthesis in the liver is quantitatively much less important than previously thought. Conversely many extrahepatic tissues may synthesize *in situ* much of the cholesterol that they require for cholesterol turnover (Spady and Dietschy, 1983).

The activity of hepatic HMG-CoA reductase is controlled by the nutritional and physiological state of animals (Siperstein and Fagan, 1983; Rodwell et al., 1973, 1976). One of the nutritional factors controlling the activity of this enzyme in the liver is the amount and nature of dietary fat (Craig et al., 1972). Also, intestinal cholesterol synthesis is regulated mainly by fat absorption of dietary fat (Ide et al., 1978), serum cholesterol level (Kritchevsky, 1976, 1977), and the nutritional state of animals (Strandberg et al., 1983), bile acid flux, intracellular cholesterol level (Shefer et al., 1973) and cholesterol itself in a negative feedback manner (Frantz et al., 1954; Brown et al., 1974). Furthermore, it has been reported that the hepatic and intestinal HMG-CoA reductase activities depended on various source of dietary fats (Bochenek and Rodgers, 1979). However, most of these studies have employed rats fed various experiment diets. There are few

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studies on the relationship between dietary fats and cholesterol synthesis in chicks. The present study, therefore, was designed to evaluate the effect of the different types of dietary fats on activities of the hepatic and intestinal HMG-CoA reductase in chicks.

Materials and Methods

Animals and diets

Experiment 1 :

Day-old male chicks (broiler) obtained from a local hatchery were used in this experiment. At 3 weeks of age, all chicks were weighed individually and divided into four groups of 10 chicks each. Chicks were housed in portable wire battery cages where *ad libitum* supply of feed and water were freely accessible. Room temperature of 25 ± 3℃ and photoperiod of 14 hr were maintained. The experimental period was for 10 days. Table 1 shows the composition of isonitrogenous and isocaloric experimental diets, while the fatty acid composition of olive oil, safflower oil, tallow and coconut oil are shown in table 2.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS (EXPERIMENT 1)

Ingredient	Experimental treatments			
	Olive oil	Safflower oil	Tallow	Coconut oil
		(g/100 g)		
Casein mix[a]	23.0	23.0	23.0	23.0
Sucrose	36.0	36.0	36.0	36.0
Fat (olive oil)	6.0	–	–	
(safflower oil)	–	6.0		–
(tallow)	–	–	6.0	–
(coconut oil)	–	–	–	6.0
Cellulose	26.83	26.83	26.83	26.83
Mineral mix[b]	6.0	6.0	6.0	6.0
Vitamin B mix[c]	1.0	1.0	1.0	1.0
Vitamin AD mix[d]	1.0	1.0	1.0	1.0
Choline-HCl	0.17	0.17	0.17	0.17
Total	100.00	100.00	100.00	100.00
Crude protein (%)	22.0	22.0	22.0	22.0
ME (kcal/100 g)	306.6	306.6	306.6	306.6

[a] Casein 90.9, L-arginine 5.4, DL-methionine 1.7, Glycine 2.0 (mg/100 mg mixture).
[b] NaCl 14.1, $K_2HPO_4 \cdot 3H_2O$ 27.2, $CaHPO_4 \cdot 2H_2O$ 21.8, $CaCO_3$ 25.22, $MgSO_4 \cdot 7H_2O$ 8.6, $FeSO_4 \cdot 7H_2O$ 2.3, $MnSO_4 \cdot 7H_2O$ 0.665, $ZnSO_4$ 0.023, $CuSO_4 \cdot 5H_2O$ 0.025, KI 0.067 (mg/100 mg mixture).
[c] Thiamine-HCl 1,200, Riboflaven 120, Niacin 1,000, Pyridoxine-HCl 80, Ca-pantothenate 400, Folic acid 50, Cyanocobalmin 0.5, Lactose 91,893.5 (mg/100 mg mixture).
[d] Vitamin A 5,000, vitamine D_3 1,000 (IU/g mixture).

Experiment 2 :

Day-old male Single Comb White Leghorn chicks were used in the experiment. When the chicks reached 4 weeks of age, they were weighed individually and divided into five groups of 5 chicks each. The experimental diets used different chain length of fatty acid (tricaprylin, trilaurin, tripalmitin or tristearin) as dietary fats. Animals were fed experimental diets for 14 days. The composition of experimental diet and the fatty acids composition of triglyceride examined are shown in table 3.

General procedure

At the end of the experiment 1 and 2, all chicks were weighed individually and then killed by decapitation. The liver and small intestine were immediately removed and flushed with an ice-cold

TABLE 2. COMPOSITION OF FATTY ACIDS PRESENTS IN THE OLIVE OIL, SAFFLOWER OIL TALLOW AND COCONUT OIL

Fatty acids	Olive	Safflower	Tallow	Coconut
		(%)		
<12:0	–	–	–	16.0
Lauric acid		–	–	47.4
Myristic acid	–	0.2	3.3	18.0
Palmitic acid	14.0	12.3	26.2	8.0
Palmitoleic acid	1.3		–	–
Stearic acid	2.6	1.8	22.4	2.8
Oleic acid	74.0	11.2	45.3	5.6
Linoleic acid	8.1	74.3	1.6	1.6
Linolenic acid	–	0.2	0.5	–

TABLE 3. COMPOSITION OF EXPERIMENTAL DIETS (EXPERIMENT II)

Ingredient	Experimental treatments				
	Fat-free	Tricaprylin	Trilaurin	Tripalmitin	Tristearin
			(g/100 g)		
Casein mix[a]	21.0	21.0	21.0	21.0	21.0
Sucrose	55.7	43.7	43.7	43.7	43.7
Fat (tricaprylin)		5.0	–	–	–
(trilaurin)	–	–	5.0	–	–
(tripalmitin)	–	–	–	5.0	–
(tristearin)		–	–	–	5.0
Cellulose	15.13	22.13	22.13	22.13	22.13
Mineral mix[b]	6.0	6.0	6.0	6.0	6.0
Vitamin B mix[c]	1.0	1.0	1.0	1.0	1.0
Vitamin AD mix[d]	1.0	1.0	1.0	1.0	1.0
Choline-HCl	0.17	0.17	0.17	0.17	0.17
Total	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	18.5	18.5	18.5	18.5	18.5
ME (kcal/100 g)	287	287	287	287	287

[a,b,c,d] were indicated to table 1.

saline. Livers were homogenized in an ice-cold 0.25 M sucrose solution containing 1 mM ethylendiaminetetra acetate-2Na (EDTA-2Na), after which the homogenates were centrifuged (Model RS-18, Tomy Seiko) at 600xg at 4℃ for 15 min. The supernatants were recentrifuged (Model 65 P, RP40-705 rotor, Hitachikoki) at 10,500 × g at 4℃ for 60 min and the resulting precipitates (microsomal fraction) were rehomogenized in 0.25 M sucrose solution containing 10 mM dithiothreitol (DTT) and trypsin inhibitor (1 mg/7.5 ml), this enzyme solution was used for assaying the HMG-CoA reductase activity.

The small intestine was divided into two segments of equal length (about 20 cm). The proximal (from the Meckel's diverticulm to pancreatic duct area) and distal (from ileo-cecal junction to Meckel's diverticulm side) are referred to as the jejunum and ileum, respectively. Each intestinal segment was flushed thoroughly with ice-cold saline and cut open. Epithelial cells were isolated by the dual-buffer method to minimize the enzyme impairment during the isolation procedure (Sugano et al., 1978). The method

initially described by Weiser (1973) and modified by Merchant and Heller (1977) was further modified as follows. The segments were cut open and immersed in 10 ml of Weiser's buffer A kept at 37℃ with gentle shaking (45 stroking per minute) on metabolic shakshaker. After 15 min, the segments were immersed in 10 ml of Weiser's buffer B for 60 min at 37℃ to obtain epithelial cells. Released epithelial cells were sedimented by centrifugation at 1,500xg for 20 min and homogenized in potassium phosphate buffer pH 7.2 containing 20 mM EDTA and the 10 mM DTT. The protein content of solution used for enzyme assay was determined by the method of Lowry et al. (1951). Enzyme activities are expressed as picomole of substrate converted to product per minute per mg protein at 38℃. The contents of various lipid fractions in the liver were analyzed by the method of Tanaka et al. (1979).

The data were analyzed using the one-way layout design of the analysis of variance. Significant differences among treatments were determined using Duncan's multiple range (Duncan, 1952).

Results

Experiment 1 (Effect of dietary fat saturation) :

The body weight, feed intake and liver weight in chicks fed experimental diets are shown in table 4. Chicks fed the safflower oil-containing diet had a significantly lower body weight gain than those fed the other oil-containing diets. None of the dietary fats significantly affected the liver weight (g/100 g).

TABLE 4. BODY AND LIVER WEIGHTS IN CHICKS FED VARIOUS DIETARY FATS (EXPERIMENT 1)

Diet	I.B.W.[1]	B.W.G.[2]	Liver Weight	
	(g)	(g)	(g)	(%)
Olive oil	638 ± 7[3]	425 ± 22[b]	25.1 ± 1.0[b]	2.36 ± 0.06
Safflower oil	638 ± 5	309 ± 8[a]	20.0 ± 0.8[a]	2.12 ± 0.08
Tallow	638 ± 4	452 ± 34[ab]	24.5 ± 1.4[b]	2.25 ± 0.07
Coconut oil	638 ± 6	489 ± 15[b]	24.0 ± 0.8[b]	2.13 ± 0.07
p[4]	NS[5]	< 0.01	< 0.01	NS

[1] I.B.W.: Inittial body weight.
[2] B.W.G.: Body weight gain.
[3] Values are mean ± S.E. for 10 chicks.
[4] Probability of a significant treatment effect.
[5] NS = *not significant.*
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.01$).

Table 5 shows the hepatic and intestinal HMG-CoA reductase activities in chicks fed diets containing different types of dietary fats. The hepatic HMG-CoA reductase activity in chicks fed the tallow-containing diet was higher than those fed the other oil-containing diets, whereas the intestinal HMG-CoA reductase activity showed the highest value in chick fed the olive oil-containing diet. When chicks were fed the safflower or coconut oil-containing diet, the intestinal HMG-CoA reductase activity was significantly low in comparison with the olive oil-containing diet.

Table 6 shows the effect of different types of dietary fats on various lipid fractions in the serum and liver of chicks. The serum cholesterol ester concentration was significantly higher in chicks fed the diet containing olive or safflower oil than in those fed the coconut oil-containing diet. Chicks fed the diet containing olive oil had significantly higher serum free cholesterol and triglyceride concentrations than those fed diets containing the other oils. The serum phospholipid concentration in chicks fed the olive oil-containing diet was significantly higher than chicks fed coconut oil or tallow. The hepatic free cholesterol content was significantly lower in chicks fed the safflower oil-containing diet than the other oil

TABLE 5. HEPATIC AND INTESTINAL HMG-CoA REDUCTASE ACTIVITIES IN CHICKS FED VARIOUS DIETARY FAT (EXPERIMENT I)

Diet	Liver	Small intestinal
	(picomole/min/mg/protein[1])	
Olive oil	50.7 ± 2.3[2a]	2.5 ± 0.2[b]
Safflower oil	50.6 ± 6.2[a]	1.5 ± 0.2[a]
Tallow	73.2 ± 7.2[b]	1.8 ± 0.3[ab]
Coconut oil	47.5 ± 7.2[a]	1.6 ± 0.2[a]
p[3]	< 0.01	< 0.05

[1] Activities expressed as substrate converted to product per minute per mg protein at 38℃.
[2] Values are mean ± S.E. for 10 chicks.
[3] Probability of a significant treatment effect.
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.01$, $p < 0.05$).

treatments. The hepatic triglyceride concentration in chicks fed the olive oil-containing diet was significant higher than in chicks fed other oil-containing diets. When coconut oil was added to the diet, the hepatic phospholipid content showed the highest level among the dietary treatments, and there was a significant difference between coconut and safflower oils.

Experiment 2 (Effect of chain length of dietary fatty acids) :

Table 7 shows body weight, feed intake and liver weight in chicks fed diets containing fatty acid sources with different chain lengths. Feed intakes were lower when the fat-free or tricaprylin-containing diet was fed than when the other diets were fed. Body weight gains were significantly lower when the fat-free diet was fed than when fat-containing diets were fed. The liver weight (g/100 g body weight) was greater in chicks fed the fat-free or tricaprylin-containing diet than in those fed diets containing any other triglycerides.

TABLE 6. THE CONTENTS OF VARIOUS LIPID FRACTIONS IN THE LIVER AND SERUM OF CHICKS FED VARIOUS DIETARY FATS (EXPERIMENT I)

Diet	Cholesterol ester	Triglyceride	Free cholesterol	Phospholipid
Serum		(mg/100 ml)		
Olive oil	280.6 ± 10.1[1b]	132.9 ± 17.7[b]	124.7 ± 9.6[b]	706.9 ± 47.9[c]
Safflower oil	270.1 ± 15.0[b]	65.1 ± 8.2[a]	97.8 ± 5.9[a]	617.4 ± 33.5[bc]
Tallow	233.6 ± 21.7[ab]	80.8 ± 15.9[a]	92.9 ± 7.8[a]	547.3 ± 34.6[ab]
Coconut oil	202.7 ± 17.7[a]	53.4 ± 6.7[a]	75.9 ± 3.4[a]	484.4 ± 39.1[a]
p[2]	< 0.05	< 0.01	< 0.05	< 0.05
Liver		(mg/g)		
Olive oil	–	19.1 ± 4.4[b]	5.0 ± 0.3[b]	51.3 ± 1.7[ab]
Safflower oil	–	9.5 ± 3.4[a]	3.7 ± 0.5[a]	47.3 ± 1.3[a]
Tallow		7.7 ± 2.0[a]	5.2 ± 0.2[b]	50.0 ± 1.3[ab]
Coconut oil	–	7.9 ± 1.4[a]	5.4 ± 0.2[b]	53.9 ± 1.4[b]
p[2]		< 0.01	< 0.05	< 0.05

[1] Values are mean ± S.E. for 10 chicks.
[2] Probability of a significant treatment effect.
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.01$, $p < 0.05$).

Table 8 shows the effect of dietary fat chain length on the HMG-CoA reductase activities of the liver, jejunum and ileum in chicks. The hepatic HMG-CoA reductase activity was significantly higher when fat-free or trilaurin-containing diet were fed than when the other triglycides were fed. The jejunal HMG-CoA reductase activity also tended to be higher when trilaurin-containing diet was fed than when the other triglycerides-containing diets were fed. The ileal HMG-CoA ruductase activity showed a significantly higher level in chick fed the trilaurin or tripalmitin-containing diet as compared to the other treatments.

Table 9 shows the effect of the dietary fat

TABLE 7. BODY AND LIVER WEIGHTS AND FEED INTAKE IN CHICKS FED DIETARY FAT WITH DIFFERENT CHAIN LENGTHS (EXPERIMENT Ⅱ)

Diet	I.B.W.[1]	B.W.G[2]	Feed intake	Liver	Weight
	(g)	(g)	(g/day/chick)	(g)	(%)
Fat-free	420 ± 13[3]	80 ± 2[a]	37.0	16.6 ± 0.3	3.81 ± 0.21[b]
Tricaprylin	420 ± 13	139 ± 5[b]	34.9	15.7 ± 1.1	3.56 ± 0.19[b]
Trilaurin	420 ± 13	232 ± 10[c]	44.3	16.1 ± 0.6	2.77 ± 0.17[a]
Tripalmitin	420 ± 11	206 ± 13[c]	43.3	16.9 ± 1.0	3.20 ± 0.21[ab]
Tristearin	420 ± 11	199 ± 15[bc]	41.1	16.9 ± 0.7	3.22 ± 0.15[ab]
p[4]	NS[5]	< 0.01		NS	< 0.05

[1] I.B.W.: Initial body weight.
[2] B.W.G.: Body weight gain.
[3] Values are mean ± S.E. for 10 chicks.
[4] Probability of a significant treatment effect.
[5] NS = not significant.
[a,b,c] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.01$, $p < 0.05$).

TABLE 8. HEPATIC AND INTESTINAL HMG-COA REDUCTASE ACTIVITIES OF CHICKS FED DIETARY FAT WITH DIFFERENT CHAIN LENGTHS (EXPERIMENT Ⅱ)

Diet	Liver	Small intestine	
		Jejunum	Ileum
		(picomole/min/mg/protein[1])	
Fat-free	11.0 ± 0.3[2c]	0.55 ± 0.12	1.24 ± 0.23[a]
Tricaprylin	7.9 ± 0.2[b]	0.72 ± 0.10	1.20 ± 0.27[a]
Trilaurin	11.0 ± 0.1[c]	0.91 ± 0.20	2.14 ± 0.24[b]
Tripalmitin	7.8 ± 0.1[b]	0.58 ± 0.09	1.90 ± 0.26[b]
Tristearin	6.6 ± 0.1[a]	0.76 ± 0.15	1.52 ± 0.24[a]
p[3]	< 0.05	NS[4]	< 0.05

[1] Activity expressed as substrate converate to product per minute per mg protein at 38℃.
[2] Values are mean ± S.E. for 5 chicks.
[3] Probability of a significant treatment effect.
[4] NS = not significant.
[a,b,c] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

chain length on the contents of the various lipid fractions of the serum and liver in chicks. The concentrations of esterified and free cholesterol in the serum were significantly higher when the fat-free diet was fed than when the fat-containing diets were fed. Furthermore, these concentrations were higher when the tricaprylin-containing diet was fed than when any other triglycerides were fed. The serum triglyceride concentration in chicks fed the far-free diet showed the highest level among treatments and there were a significant difference between the fat-free and the trilaurin or tripalmitin-containing diets. The serum phospholipid concentration showed the highest level in chicks fed the fat-free diet, and there was a significant difference between the fat-free and triglyceride-containing diets. The hepatic triglyceride content were significantly higher in chicks fed the fat-free or tristearin-containing diet than in those fed other triglyceride-containing diet. Differences were not significant for the hepatic free cholesterol content among the dietary treat-

TABLE 9. THE CONTENTS OF VARIOUS LIPID FRACTIONS IN THE LIVER AND SERUM OF CHICKS FED DIETARY FAT WITH DIFFERENT CHAIN LENGTHS (EXPERIMENT II)

Dietary	Cholesterol ester	Triglyceride	Free cholesterol	Phospholipid
Serum		(mg/100 ml)		
Fat-free	256 ± 18[1c]	58.6 ± 6.3[b]	51.4 ± 2[c]	404 ± 22[b]
Tricaprylin	204 ± 7[b]	41.1 ± 11[ab]	44.0 ± 3[bc]	284 ± 16[a]
Trilaurin	156 ± 3.8[a]	30.2 ± 5.7[a]	32.6 ± 0.4[a]	296 ± 11[a]
Tripalmitin	162 ± 5[a]	24.2 ± 4.2[a]	38.4 ± 5.1[ab]	286 ± 18[a]
Tristearin	153 ± 4.2[a]	43.2 ± 8.8[ab]	36.6 ± 1.8[ab]	304 ± 9.3[b]
p[2]	< 0.05	< 0.05	< 0.05	< 0.05
Liver		(mg/g)		
Fat-free	–	4.74 ± 0.76[b]	3.04 ± 0.09	36.4 ± 1.7[a]
Tricaprylin		2.25 ± 0.76[a]	3.05 ± 0.09	40.4 ± 1.6[ab]
Trilaurin	–	2.54 ± 0.26[a]	3.24 ± 0.03	43.1 ± 1.4[b]
Tripalmitin	–	2.05 ± 0.24[a]	3.12 ± 0.13	39.8 ± 1.2[ab]
Tristearin		5.26 ± 0.7[b]	2.94 ± 0.07	37.5 ± 2.4[ab]
p[2]	–	< 0.05	< 0.05	< 0.05

[1] Values are mean ± S.E. for 5 chicks.
[2] Probability of a significant treatment effect.
[a,b,c] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

ments. The hepatic phospholipid content showed the highest level in chicks fed the trilaurin-containing diet and there was a significant between difference the trilaurin-containing and fat-free diets. Table 10 shows the effect of the dietary fat length on contents of various lipid fractions

TABLE 10. THE CONTENTS OF VARIOUS LIPID FRACTIONS IN THE JEJUNUM AND ILEUM OF CHICKS FED DIETARY FAT WITH CHAIN LENGTHS (EXPERIMENT II)

Diet	Triglyceride	Free cholesterol	Phospholipid
Jejunum		(mg/g)	
Fat free	38.0 ± 4.2[1a]	109.8 ± 3.7[bc]	840 ± 10.9[a]
Tricaprylin	33.0 ± 2.1[a]	68.8 ± 11.4[a]	458 ± 9.9[b]
Trilaurin	56.4 ± 5.2[b]	107.2 ± 5.4[bc]	812 ± 24.4[a]
Tripalmitin	41.4 ± 4.6[a]	85.6 ± 7.2[ab]	810 ± 69.6[a]
Tristearin	38.4 ± 5.2[a]	91.6 ± 11.1[abc]	868 ± 12.5[a]
p[2]	< 0.05	< 0.05	< 0.05
Ileum			
Fat-free	72.0 ± 16.4[bd]	48.6 ± 5.2[b]	274 ± 23
Tricaprylin	96.2 ± 12.2[cd]	38.8 ± 2.8[ab]	268 ± 25
Trilaurin	33.7 ± 5.7[a]	41.6 ± 3.2[ab]	288 ± 30
Tripalmitin	128.8 ± 9.2[cd]	31.2 ± 3.2[a]	193 ± 29
Tristearin	47.1 ± 9.1[ab]	39.6 ± 4.1[b]	294 ± 50
p[2]	< 0.05	< 0.05	NS[3]

[1] Values are mean ± S.E. for 5 chicks.
[2] Probability of a significant treatment effect.
[3] NS = not significant.
[a,b,c,d] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

of the jejunum and ileum in chicks. The triglyceride content of the ileum was higher than that of the jejunum. On the other hand, the phospholipid content of the jejunum was higher than that of the ileum. The jejunal triglyceride content was significantly higher when the trilaurin-containing diet was fed than when diets containing any other triglycerides were fed. The jejunal of free cholesterol and phospholipid contents were significantly, or tended to be lower, when the tricaprylin-containing diet was fed than when other experimental diets were fed. The ileal triglyceride contents in chicks fed the tripalmitin or trilaurin-containing diet showed the highest or lowest value among the experimental diets, respectively. The ileal free cholesterol content was significantly lower or tended to be lower when the tripalmitin-containing diet was fed than when any other experimental diets were fed. The ileal phospholipid content in chicks fed the tripalmitin-containing diet showed the lowest value, although a statistically significant difference was not observed among experimental treatments.

Discussion

These studies were carried out in the expectation that the direct assay of HMG-CoA reductase which catalyzed the rate-limiting step of the hepatic and intestinal cholesterol synthesis might provide more precise measurements of synthetic rates than previous estimates based on acetate incoporation.

In the small intestinal mucosa of chicks, the HMG-CoA reductase activity predominates in the ileum and is considerably lower in the jejunum (Experiment 2). Our finding are in poor agreement with the observations in rabbits or rats. (Stange et al., 1981; Sugano et al., 1982 and Oku et al., 1985).

Cholesterol synthesis in the small intestinal mucosa is confined to the area of the epithelial cell generation, suggesting that it serves the function of providing structural cholesterol for newly forming cells (Dietschy and Siperstein, 1967). Hence the increased demand for newly synthesized cholesterol in the ileum in response to dietary fat may be due to increased cell renewal (Dietschy and Siperstein, 1965). In the small intestinal mucosa of the chick increased cholesterol synthesis for the formation of portmicron coat is required for the transport of absorbed fat to the portal vein, particularly, when dietary cholesterol was not given as in the present study. The hepatic HMG-CoA reductase activity is known to be influenced by the degree of dietary fat saturation (Ide et al., 1978). Shefer et al., (1972) have reported that the extent of saturation of dietary fat affected HMG-CoA reductase activities of the liver and small intestine in rats. In the experiment 1, the hepatic and intestinal HMG-CoA reductase activities were, also, influenced by the degree of dietary fat saturation. The intestinal HMG-CoA reductase activity showed the highest value in chicks fed the olive oil-containing diet. Although olive oil (Plant oil) and tallow (animal fat) have the same saturation, HMG-CoA reductase activities of the liver and small intestine were different in chicks fed those oils. The hepatic HMG-CoA reductase activity was lower in chicks fed the olive oilcontaining diet than in those fed the tallow-containing diet (experiment 1). Fats of animal origin usually contain cholesterol, whereas vegetable oils contain plant sterols. As the plant sterols are not readily absorbed from the intestine, the action of plant sterols in cholesterol metabolism has been attributed to the inhibition of cholesterol absorption in the gut (Sim et al., 1980). A low cholesterol absorption in the small intestine would be relevant to higher intestinal and lower hepatic HMG-CoA reductase activities in chicks fed the olive oil-containing diet. Stange et al. (1983) have reported that the principal regulator of intestinal cholesterogensis may be the cellular cholesterol balance determined by the influx and efflux of cholesterol. Thus, increased HMG-CoA reductase activity of the ileum in rats fed long-chain saturated fats reflects the changes in either one or both of these two parameters. The influx into the epithelum is mainly affected by cholesterol levels of the serum and diet (Anderson and Dietschy, 1977). In the experiment 2, the serum esterified and free cholesterol concentrations were higher when the tricaprylin-containing diet was fed than when any other triglyceride were fed, whereas the ileal HMG-CoA reductase activity was low when chicks were fed this diet. Furthermore, the serum free cholesterol concentration was lower when trilaurin was fed than when any other triglyceride were fed, although the jejunal and ileal HMG-CoA reductase activities showed the highest value in

chicks fed the trilaurin-containing diet. Gelhard and Prigge (1981) studied the intestinal HMG-CoA reductase activity during perfusion of fatty acids through isolated ileal segments in dogs. Except for hexanoic acids, all the fatty acids studied showed a stimulator effect and the degree of stimulation appeared to be dependent on neither saturation nor chain length. However, the stimulation of the intestinal epithelium was higher when the trilaurin-containing diet was fed than when any other triglyceride were fed. The stimulation by trilaurin of the intestinal HMG-CoA reuctase activity is, therefore, not solely explained by cholesterol balance in intestinal cells. Alfin et al. (1965) have suggested that the methyl ester of lauric acid accentuates essential fatty acid deficiency signs compared to long chain saturated esters. Furthermore, essential fatty acid deficiency appears to affect the synthesis or release of chylomicron lipid by the intestine (Clark et al., 1973). As described for Alfin et al. (1965) using rats, the stimulation by trilaurin may be related to an aggravated essential fatty acid deficiency in the intestinal epithelium, since linoleic acid was not included in this experimental diet. Activities of the hepatic and intestinal HMG CoA reductase were easily modulated by dietary fat manipulations. However, the mechanism by which these activities is regulated by different fats is presently uncertain.

In this study, jejunal and ileal cholesterol contents were higher in chicks fed the trilaurin-containing diet than in those fed diets containing any of triglycerides. Hence, a definite correlation between cholesterol content and HMG-CoA reductase activity of the small intestine was not observed. Furthermore, cholesterol contents of the liver and serum did not always correlate with the hepatic and intestinal HMG-CoA reductase activities.

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Literature Cited

Alfin-Slater, R. B., R. S. Morris, H. Hanson and J. F. Proctor. 1965. Effect of non-essential fatty acid deficiency. J. Nutr. 87:168-172.

Anderson, J. M. and J. M. Dietschy. Regulation of sterol synthesis in 15 tissue of rat, 2. Role of rat and human high and low density lipoprotein and of rat chylomicron remnants. J. Biol. Chem. 252: 3652-3659.

Bochenek, W. S. and J. B. Rodgers. 1979. Dietary regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in villous and crypt cells of the rat small intestine. Biochem. Biophys. Acta. 75:57-62.

Brown, H. S., S. E. Dana and J. L. Goldstein. 1974 Regulation of 3-hydroxy 3 methylglutaryl coenzyme A reductase activity in cultured human fibroblasts. J. Biol. Chem. 249 789-796.

Clark, S. B., T. E. Ekkers, A. Signh, J. A. Balint, P. R. Holt and J. B. Rodgers. 1973. Fat absorption in essentia. fatty acid deficiency: a model experimental approach to studies of the mechanism of fat malabsorption of unknown etiology. J. Lipid Res. 14:581-588.

Craig, M. C., R. E. Dugan, R. A. Muesing, L. L. Slakey and J. W. Porter 1972. Comparative effects of dietary regimens on the levels of enzymes regulating the synthesis of fatty acids and cholesterol in rat liver. Arch. Biochem. Biophys. 151:128-136.

Dietschy, J. M. and M. D. Siperstein 1965. Cholesterol synthesis by the gastrointestinal tract: localization and mechanism of control. J. Clin. Invest. 44:1311-1327.

Dietschy, J. M. and M. D. Siperstein. 1967. Effect of cholesterol synthesis in seventeen tissues of the rat. J. Lipid Res. 8:97-104.

Dietschy, J. M. and J. D. Wilson. 1968 Cholesaterol synthesis in the squirrel monkey relative rates of synthesis in various tissues and mechanism of control. J. Clin. Invest. 47:166-174.

Duncan, D. B. 1952 Mutiple range and multiple F test. Biometric. 11 1-42.

Feedman, F. B., B. S. Russell, F. H. Schnare, I. Moreti-RoJas, B. S. Miles and E. A. Dolyle. 1979. Effects of diets of homogeneous saturated triglycerides on cholesterol balance in rats. J. Nutr. 109: 2237-2246.

Frantz, I. D., H. S. Schneider and B. T. Hinkelmen. 1954. Supperssion of hepatic cholesterol synthesis in the rat by cholesterol faading. J. Biol. Chem. 206:465-469.

Gelhard, R. L. and W. F. Prigge 1981 *In vivo* regulation canine intestinal 3-hydroxy-3-methylglutaryl coenzyme reductase by cholesterol lipoprotein and fatty acids. J. Lipid Res. 22:1111-1118.

Ide, T., H. Okamatsu and M. Sugano. 1978. Regulation by dietary fats of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in rat liver. J. Nutr. 108: 601-612.

Kritchevsky, D. 1976. Diet and atherosclerosis. Am. J. Pathol. 84:615-632.

Kritchevsky, D. 1977. Dietary fiber and other dietary factors in hypercholesterolemia. Am. J. Clin. Nutr.

30:979-984.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Bio. Chem. 193:265-275.

Merchant, J. L. and R. A. Heller. 1977. 3-hydroxy-3-methylglutaryl coenzyme A reductase in isolated villous and crypt cells of the rat ileum. J. Lipid Res. 18:727-733.

Oku, H. and M. Sugano. 1985. Dietary fat dependence of intestinal 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in rat. J. Nutr. 115:880-889.

Shefer, S., S. Hauser, V. Lapar and E. H. Mosbach. 1972. HMG-CoA reductase of intestinal mucosa and liver of rat. J. Lipid Res. 13:402-412.

Shefer, S., S. Hanser, I. Lapar and E. H. mosbch. 1973. Regulatory effects of dietary sterols and bile acids on rat intestinal HMG-CoA reductase. J. Lipid Res. 14:400-405.

Sim, J. S., W. D. Kitts and D. B. Bragg. 1980. Influence of dietary oil, cholesterol and soysterols on the fecal natural and acidic steroid excretion in laying hens. Poult. Sci. 59:325-327.

Siperstein, V. W. and V. W. Fagan. 1964. Studies on the feedback regulation of cholesterol synthesis. Adv. Enz. Reg. 2:249-264.

spady, D. K. and J. M. Dietschy. 1983. Sterol synthesis *in vivo* in 18 tissues of the squirrel monkey, guinea pig, rabbit and rat. J. Lipid Res. 24:303-315.

Stange, E. F., K. E. Sucking and J. M. Dietschy. 1983. Synthesis and coenzyme A-dependent esterification of cholesterolin rat intestinal epithelium. J. Bic. Chem. 258:12868-12875.

Stange, E. F., M. Alavi, A. Schneider, H. Ditschuneit and J. R. Poley. 1981. Influence of dietary cholesterol, saturated and unsaturated lipid on 3-hydroxy-3-methylglutaryl coenzyme A reductase and sterol synthesizing activity in rabbit intestine and liver. J. Lipid Res. 22:47-56.

Strandberg, T. E., R. S. Tilvis and T. A. Miettinen. 1983. Regulation of cholesterol synthesis in jejunal absorptive cells of rat. Scand. J. Gastroenterol. 18:1017-1023.

Sugano, M., H. Okamats and T. Ide. 1978. Properties of 3-hydroxy-3-methylglutaryl coenzyme A reductase in villous and crypt cells of the rat small intestine. Agric. Biol. Chem. 42:2009-2014.

Sugano, M., Y. Fujisaki, U. Oku and T. Ide. 1982. 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in the small intestine of rats fed non-purified and semipurified diets. J. Nutr. 112:51-59.

Tanaka, K., K. Kitahara and K. Shigero. 1979. Effect of dietary protein level on lipid metabolism in growing chicks. Jpn. J. Zootech. 50:44-54.

Tarley, S. D., J. M. Anderson and J. M. Dietschy. 1981. Rate of sterol synthesis and up take in the major organs of the rat *in vivo*. J. Lipid Res. 22:551-569.

Weiser, M. M. 1973. Intestine epithelial cell surface membrane glycoprotein synthesis. J. Biol. Chem. 248:2536-2541