THE MECHANISM OF HYPERCHOLESTEREMIA INDUCED BY THE INGESTION OF FAT

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INTRODUCTION

The cause of the increase of serum cholesterol by the ingestion of saturated fatty acids and the invariability of that by the ingestion of polyunsaturated fatty acids are not yet clear.

The change of serum cholesterol seems from the theory to be due to the following four causes: 1) promotion and inhibition of cholesterol degradation to bile acids, 2) transfer of circulating cholesterol rich lipoprotein to another body pool, 3) promotion and inhibition of cholesterol synthesis, 4) restriction of cholesterol intake or interference with its absorption or reabsorption.

Lewis *et al*¹⁻³ described that the diet rich in polyunsaturated fatty acids promoted the excretion of bile acid. However, Spritz *et al*⁴ described that the excretion of bile acids did not increase and the transfer of circulating cholesterol might concern with the change of the serum cholesterol. Kinsell *et al*⁵ reported that the serum cholesterol lowering action of polyunsaturated fatty acids (the action to maintain the normal level of the serum cholesterol) might be due to the inhibition of the synthesis of cholesterol.

Recently, Tidwell *et al*⁶ reported that the diets rich in saturated fatty acids promoted the cholesterol synthesis from acetic acid in the liver. We investigated whether the ingestion of saturated fatty acids and polyunsaturated fatty acids concerned the degradation and synthesis of cholesterol using rats, and will report that results.

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EXPERIMENT

1. Male rats weighing about 200 g were divided into 10 groups consisting of 5 rats in a group. The rats from the first to the fifth groups were fed by the cholesterol palmitic acid diet and from the sixth to the tenth groups by the cholesterol linoleic acid diet. After feeding for two weeks, the rats from the first to the fifth groups were administered $9.5\ \mu\text{C}$ — $12.5\ \mu\text{C}$ of 1-C^{14} palmitic acid (3.90×10^5 c.p.m.— 5.12×10^5 c.p.m.) dissolved in 1.9 g—2.5 g of palmitic acid and 0.4 g—0.5 g of cholesterol simultaneously. Saturated fatty acids and unsaturated fatty acids of the serum cholesterol esters in the liver were determined using of our method⁷ after 3 hours following administration in the first group, 6 hours in the second group, 12 hours in the third group, 24 hours in the fourth group and 48 hours in the fifth group, and their specific activities were determined by a gas G.M. counter.

Cholesterol Palmitic Acid Diet

Kasein*	14 g	D ₃	400 I. U.
Sucrose	39 g	Ascorbic acid	75 mg
Starch	40 g	Pyridoxine	3 mg
M. Salz	4 g	α -Tocopherol	2 mg
Palmitic acid	1.5 g	B ₁₂	0.002 mg
Cholesterol	1.5 g	Niacin	20 mg
A	3,000 I. U.	Pantothenic acid	1 mg
Thiamin-HCl	2 mg	K	0.4 mg
B ₂	3 mg	Choline	0.1 g

* Whashed by the warmed methanol and ether.

Linoleic acid was used for the cholesterol linoleic acid diet in place of palmitic acid.

The rats from the sixth to the tenth groups were administered $7.1\ \mu\text{C}$ — $12.5\ \mu\text{C}$ (3.43×10^5 c.p.m.— 6.04×10^5 c.p.m.) of 1-C^{14} linoleic acid dissolved in 1.4 g—2.5 g of linoleic acid and 0.3 g—0.5 g of cholesterol simultaneously. The determination of specific activities of fatty acids of the serum cholesterol esters in the liver was carried out as the same procedure as that described above, after 3 hours following administration in the sixth group, 6 hours in the seventh group, 12 hours in the eighth group, 24 hours in the ninth group and 48 hours in the tenth group.

2. Rats were divided into 18 groups in a similar fashion of the above experiment. Cholesterol was removed from the above mentioned palmitic acid diet. The first group was fed by the diet with palmitic acid in 10% and

administered by $9.0\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose. The second group was fed by the diet with oleic acid in 10% and administered $6.6\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose. The third group was fed by the diet with linoleic acid in 10% and administered $5.4\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose. The fourth group was administered $5.4\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} palmitic acid dissolved in 10% palmitic acid. The fifth group was administered $6.3\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} oleic acid dissolved in 10% oleic acid. The sixth group was administered $4.3\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} linoleic acid dissolved in 10% linoleic acid. The seventh group was administered $6.3\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose after 2 weeks of feeding with the diet with palmitic acid in 10%. The eighth group was administered $7.2\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose after 2 weeks of feeding with the diet with oleic acid in 10%. The ninth group was administered $6.0\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose after 2 weeks feeding with the diet with linoleic acid in 10%. The tenth group was administered $7.8\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} palmitic acid after 2 weeks of feeding with the diet with palmitic acid in 10%. The eleventh group was administered $8.1\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} oleic acid after 2 weeks of feeding with the diet with oleic acid in 10%. The twelfth group was administered $5.4\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} linoleic acid after 2 weeks of feeding with the diet with linoleic acid in 10%. The thirteenth group was administered $8.1\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose after 4 weeks of feeding with the diet with palmitic acid in 10%. The fourteenth group was administered $9.0\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose after 4 weeks of feeding with the diet with oleic acid in 10%. The fifteenth group was administered $5.4\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} glucose after 4 weeks of feeding with the diet with linoleic acid in 10%. The sixteenth group was administered $9.0\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} palmitic acid after 4 weeks of feeding with the diet with palmitic acid in 10%. The seventeenth group was administered $6.9\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} oleic acid after 4 weeks of feeding with the diet with oleic acid in 10%. The eighteenth group was administered $6.3\ \mu\text{C}$ — $10.0\ \mu\text{C}$ of 1-C^{14} linoleic acid after 4 weeks of feeding with the diet with linoleic acid in 10%. In each group, after 3 days following administration, the specific activity of total cholesterol in the liver and the serum to the daily dosage was determined.

3. The separation of cholesterol from the liver and the serum:

The liver or the serum was extracted with methanol-ether (3:1) and the extract was saponificated. Unsaponificated fraction was separated by petroleum-ether washed with $\frac{N}{5}$ KOH methanol solution and was subjected to chromatography of woelm neutral almina. The woelm neutral almina was

Table 1
*The change of the incorporation of the liver and the serum cholesterol ester fatty acids
 in rats administered C-14 palmitic acid or 1-C14 linoleic acid following two weeks
 feeding with the palmitic acid, linoleic acid or cholesterol diet*

Administration (per day)	Tissue	Fatty acid	Quantity (%)	Fatty acid of cholesterol-ester				
				Specific activity (average % of total fatty acid 100 mg)				
				3 hours after	6 hours after	12 hours after	24 hours after	42 hours after
1-C ¹⁴ palmitic acid 1.9 g-2.5 g (9.5 μ C-12.5 μ C) + Cholesterol 0.4 g-0.5 g	Liver	Total		5.9	8.0	6.0	8.1	1.7
		Saturated	31.2	5.8	7.9	5.9	7.9	1.7
	Serum	Unsaturated	68.8	0.1	0.1	0.1	0.2	0
		Total		8.1	17.4	4.8	7.3	1.2
1-C ¹⁴ linoleic acid 1.4 g-2.5 g (7.1 μ C-12.5 μ C) + Cholesterol 0.3 g-0.5 g	Liver	Saturated	35.5	7.7	17.4	4.7	7.0	1.2
		Unsaturated	64.5	0.4	0	0.1	0.3	0
	Serum	Total		10.8	12.7	13.8	15.7	8.0
		Saturated	29.7	1.0	0.1	0.8	2.6	1.2
	Liver	Unsaturated	70.3	9.8	11.8	13.0	13.1	6.8
		Total		35.6	52.6	25.1	26.4	8.6
	Serum	Saturated	30.3	1.1	6.7	3.6	3.2	0.8
		Unsaturated	69.7	34.5	45.9	21.5	23.2	7.8

washed with water, dried at 100°C for 6 hours and was packed into an absorption tube using petroleum-ether. The tube was eluted by 1% methanol-petroleum-ether, followed by 5% methanol-petroleum-ether for the fractionary separation.

RESULT AND DISCUSSION

The specific activity of fatty acids of cholesterol esters in the liver and the serum was determined after 3, 6, 12, 24, or 48 hours following administration of 1-C¹⁴ palmitic acid and cholesterol or 1-C¹⁴ linoleic acid and cholesterol in rats which had been fed with the cholesterol-palmitic acid diet or the cholesterol-linoleic acid diet for 2 weeks. The result is shown in Table 1.

There were no great differences of the specific activity of the total fatty acid in the serum between the administration of 1-C¹⁴ palmitic acid and that of 1-C¹⁴ linoleic acid. The values of both groups got maximum at the sixth hour and decreased in a same degree. In the liver, the decrease of the specific activity in course of time was lower in the group of 1-C¹⁴ linoleic acid than in the group of palmitic acid. Saturated fatty acids in the liver and in the serum slightly decreased in course time in the groups administered 1-C¹⁴ linoleic acid. However, the levels of unsaturated fatty acids in the liver and the serum were nearly zero in the groups administered 1-C¹⁴ palmitic acid.

Therefore, the rate of degradation of cholesterol ester in the liver was not changed by the administration of palmitic acid or linoleic acid. Fatty acids in cholesterol esters seem to be hardly subjected to unsaturation and to be subjected to saturation. We reported that unsaturated fatty acids in the liver cholesterol esters decreased larger when cholesterol-linoleate was ingested than when cholesterol-palmitate was ingested.⁸ It may be due to the saturation of fatty acids in the liver cholesterol esters.

Next, an experiment to investigate whether the change of the serum cholesterol concerned the cholesterol synthesis in the liver was carried out. Rats were fed by the diet containing palmitic acid, oleic acid and linoleic acid for 2 or 4 weeks and then administered 1-C¹⁴ glucose, 1-C¹⁴ palmitic acid, 1-C¹⁴ oleic acid and 1-C¹⁴ linoleic acid for 3 days, and the specific activities of the liver and serum cholesterol were determined. The result is shown in Table 2.

At the start, the specific activities of cholesterol in the livers and the sera of rats administered 1-C¹⁴ palmitic acid were about three times those

Table 2

*The incorporation of fatty acid with the liver and the serum cholesterol in rats administer 1-C¹⁴ glucose, 1-C¹⁴ palmitic acid, 1-C¹⁴ oleic acid and 1-C¹⁴ linoleic acid following two or four weeks feeding with the palmitic acid, linoleic acid or cholesterol diet**

Tissue	Diet (added 10%)	C ¹⁴ administration	Specific activity of cholesterol (average % of 100 mg)		
			Start	2 weeks feedings	4 weeks feedings
Liver	Palmitic acid	1-C ¹⁴ glucose	1.4	1.0	1.1
	Oleic acid	1-C ¹⁴ glucose	1.3	1.0	0.6
	Linoleic acid	1-C ¹⁴ glucose	1.0	1.2	1.3
	Palmitic acid	1-C ¹⁴ palmitic acid	3.3	8.3	4.0
	Oleic acid	1-C ¹⁴ oleic acid	2.2	8.0	3.7
	Linoleic acid	1-C ¹⁴ linoleic acid	0.7	1.4	2.1
Serum	Palmitic acid	1-C ¹⁴ glucose	1.2	0.9	1.2
	Oleic acid	1-C ¹⁴ glucose	1.5	0.8	1.7
	Linoleic acid	1-C ¹⁴ glucose	1.0	0.9	1.2
	Palmitic acid	1-C ¹⁴ palmitic acid	2.6	8.3	5.3
	Oleic acid	1-C ¹⁴ oleic acid	2.4	7.9	4.2
	Linoleic acid	1-C ¹⁴ linoleic acid	1.4	1.9	2.0

* The specific activity to the dosage per day.

of rats administered 1-C¹⁴ glucose, and those of 1-C¹⁴ oleic acid administered rats were about twice those of 1-C¹⁴ glucose administered rats. The specific activities in rats administered 1-C¹⁴ linoleic acid were less than those in rats administered 1-C¹⁴ glucose.

After 2 or 4 weeks, the specific activities in the livers and the sera of rats administered 1-C¹⁴ palmitic acid strikingly increased (two or three times), and those in rats administered 1-C¹⁴ oleic acid increased. However, the activities in rats administered 1-C¹⁴ linoleic acid or 1-C¹⁴ glucose did not increase.

It is known that cholesterol is synthesized from acetic acid.^{9,10} Acetic acid may be synthesized mainly from glucose and fatty acids. Table 2 showed that the amount of synthesized cholesterol from glucose and linoleic acid was not changed and that from palmitic acid or oleic acid increased.

Therefore, the major cause of the increase of the serum cholesterol by the ingestion of the large amount of palmitic acid may be the increase of the synthesis of cholesterol from palmitic acid (through acetic acid). The major cause that the serum cholesterol does not increase by the administration of the large amount of linoleic acid may be that the synthesis of cholesterol from linoleic acid is not promoted.

SUMMARY

The specific activities of fatty acid of cholesterol esters in the liver and the serum of rats which were administered C^{14} palmitic acid and cholesterol or C^{14} linoleic acid and cholesterol after 2 weeks feeding with the diet containing cholesterol palmitic acid or cholesterol linoleic acid were determined, and the specific activities of cholesterols in the livers and the sera of rats which were administered C^{14} glucose, C^{14} palmitic acid, C^{14} oleic acid or C^{14} linoleic acid for 3 days after 2 or 4 weeks of feeding with the diet containing palmitic acid, oleic acid or linoleic acid were also determined. There were no differences of the rates of degradation of cholesterol between rats administered palmitic acid and that administered linoleic acid. The synthesis of cholesterol was promoted in course of day by the administration of palmitic acid or oleic acid, but those from linoleic acid and glucose did not increase.

From these results, it is considered that the hypercholesteremia by the ingestion of the large amount of saturated fatty acids is mainly due to the promotion of the cholesterol synthesis from saturated fatty acids and the invariability of the serum cholesterol by the ingestion of polyunsaturated fatty acids (to maintain the normal level) is mainly due to that the cholesterol synthesis from linoleic acid does not increase.

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