

# Influence of Mg(II), Ca(II), Perilla Oil and Korean Ginseng on the Plasma Cholesterol Concentration and HMG-CoA reductase Activity of the Rabbits

Nam, Hyun-Keun

*Kwangju Health Junior College*

마그네슘, 칼슘 및 들깨 기름이 인삼 첨가 식이로 사육한 토끼의 혈액 성분 변화에 미치는 영향

남 현 근

광주보건전문대학

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## 요 약

인삼, 들깨 기름, 칼슘 및 마그네슘이 함유되어 있는 식이로 사육한 토끼의 혈장 콜레스테롤과 HMG-CoA reductase의 활성을 조사하였다.

간에 포함되어 있는 HMG-CoA reductase의 활성은 인삼과 들깨 기름 및 마그네슘 첨가 식이군에 있어서 증가되었으며 혈장 콜레스테롤의 농도는 감소되었다. 혈장 LDL-콜레스테롤은 대조군보다 감소하였으나 HDL-콜레스테롤은 증가되었다. 또한 베타 글로블린의 분포도 인삼과 마그네슘 및 들깨 기름을 첨가 급여한 실험군에 있어서 가장 낮았으며,  $\alpha$ -2-글로블린은 대조군에 비하여 가장 높은 값을 보였다. 전해질인 K<sup>+</sup>은 인삼 첨가로 감소 되었으나 Na<sup>+</sup>은 증가되었다. 혈장 마그네슘의 농도는 인삼의 첨가로 변화가 없었지만 칼슘과 들깨 기름 및 인삼 첨가식이로 마그네슘의 농도가 더 증가되었다. 혈장 칼슘의 농도는 마그네슘과 인삼을 첨가한 식이군에 있어서 감소하였다.

## I. INTRODUCTION

Many investigators indicated that there are some relationship between cholesterol concentration and arteriosclerosis. Therefor, it assumed that the higher plasma cholesterol concentration may indicate to be hypertension and arteriosclerosis (1-14). There are many factors for increasing cholesterol level such as carbohydrate, protein, lipids and others. Therefor, it is obviously related to cholesterol metabolism in the

cells which are regulated by altering dietary foods (15-21).

In previous studies (22-28), magnesium and calcium concentration may be affected to the cholesterol level in animals. Microsomal enzyme, such as Acyl-coenzyme A: Cholesterol acyltransferase (ACAT) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase control the rates of intracellular cholesterol esterification and synthesis, respectively. It is well known that these two enzymes regulate the amount of unesterified cholesterol within

a cell and responsive to changes in microsomal lipid composition (29-35).

In recent studies (36-38), many investigators indicated that the effects of various dietary fish oils on plasma lipid levels have demonstrated lowering responses. Since the fish oils contain highly unsaturated  $\omega$ -3 fatty acids compared to  $\omega$ -6 polyunsaturated fatty acids found in most vegetable and plant oils. It has also been reported that polyunsaturated fat decrease, saturated fats increase, and cholesterol has no effect on plasma triglyceride levels.

It was undertaken to evaluate the effect of commonly used dietary fat, such as perilla oil on plasma cholesterol level. The perilla oil contains C:18=3, ( $\omega$ -3) 58.3% polyunsaturated fatty acids, therefore, perilla oil reacts as a lowering factor on the plasma cholesterol levels (39-40). Some investigators indicated that Korean ginseng has a cholesterol concentration lowering act (56-58).

It assumed that the plasma cholesterol level and HMG-CoA reductase activity depend on the quantities of magnesium, calcium, perilla oil and Korean ginseng powder.

## II. MATERIALS AND METHODS

### 1. Animals

Male New Zealand white rabbits weighing 500-700g were fed basal diet for 1 week prior to the start of the experimental diets. The rabbits were divided into 7 dietary groups of two animals each.

The following diets were administered ad libitum for 30 days from May 1, 1986 to May 30, 1986. The perilla oil was analyzed by the HPLC.

### 2. Materials

Basal food was obtained from Jaeil Co., Korea. HMG-CoA, cholesterol, glucose-6-phosphate dehydrogenase, glucose-6-phosphate, and nucleotide adenine diphosphate were from Sigma chemical Co., St. Louis, M.O.

### 3. Enzyme assays

HMG-CoA reductase was determined as described (29,34).

Table 1. The experimental diet group and additive

Group	Basal diet	Additive
Control	50g B.D' only	
1	50g B.D.	1% K.G. 4 meq Mg(II)
2	50g B.D.	1% K.G. 4meq Ca (II)
3	50g B.D.	10% P.O. 4 meq Mg (II)
4	50g B.D.	10% P.O. 4 meq Ca (II)
5	50g B.D.	10% P.O. 1% K.G. 4 meq Mg(II)
6	50g B.D.	10% P.O. 1% K.G. 4meq Ca (II)

where B.D.: basal diet  
K.G.: Korea ginseng powder  
P.O.: perilla oil

Table 2. Composition of fatty acids of perilla oil

C:16	C:18	C:18=1	C:18=2	C:18=3
5.9	1.9	18.3	15.6	58.3

Table 3. The basal diet composition for rabbit (g%)

Food	Ingredient	Protein	Fat	Cholesterol
Corn powder	25	54.44	32.24	13.32
Wheat bran	15	68.54	20.04	11.42
Soybean meal	25	57.06	30.41	12.53
Wheat	25	71.78	6.89	21.33
Soybean rind	10	86.63	2.75	10.62
	100	67.69	18.47	13.84

### 4. Lipoprotein

Lipoprotein was determined by the Lipid Research Clinics Program, NIH, U.S.A. (41).

### 5. Chemical analysis

Cholesterol was determined by Schonheimer-Sperry method<sup>(42)</sup>, triglyceride by Carlson-Wadstrom method<sup>(43)</sup>, phospholipid by phosphorus assay<sup>(43)</sup>, protein fraction by electrophoresis<sup>(43)</sup> and magnesium and calcium by EDTA chelatometry<sup>(44)</sup>.

### 6. Statistical analysis

A one way analysis of variance model used to

test for significance<sup>55</sup>).

### 7. Preparation of microsomes<sup>46</sup>)

The rabbits were killed after 24hrs fasting end of the experimental periods. The liver of 2 grams were excised for the preparation of microsomes. Approximately 1 gr. of liver was diced over ice, and homogenized in a buffered sucrose solution containing 0.1M sucrose, 0.05M KCl, 0.04M  $\text{KH}_2\text{PO}_4$ , 0.03M EDTA, pH 7.4, in a homogenizer. The whole homogenates were centrifuged for 20 minutes at 10,000g and the resulting supernatants were centrifuged for 1 hr. at 105,000g. The pellets were resuspended in buffer and centrifuged again at 105,000g for 1 hr.

## III. RESULTS

### 1. Plasma lipoprotein cholesterol concentration

The plasma cholesterol response to the different dietary regimens shows Table 4. Rabbits which were fed the perilla oil plus magnesium, and perilla oil plus magnesium and korea ginseng powder for 4 weeks had had a significant decrease in their plasma total cholesterol levels compared to animals fed the control diet.

The animals fed magnesium plus perilla oil had a significant decrease in their free cholesterol concentration compared to animals fed the control diet. In the triglyceride, the animals fed korea

Table 4. Plasma lipoprotein cholesterol, triglyceride and phospholipid contents<sup>a</sup>

Group	T-CHOL	F-CHOL	E-CHOL	TG	PL
Control	108.4 ± 3	46.7 ± 5	63.7 ± 3	105.4 ± 1	175.2 ± 1
1	104.5 ± 2	56.7 ± 3	47.3 ± 3	79.4 ± 3 <sup>c</sup>	145.7 ± 2
2	114.3 ± 2	59.8 ± 2	54.2 ± 1	91.5 ± 3	133.7 ± 2
3	98.5 ± 3 <sup>b</sup>	34.4 ± 2 <sup>b</sup>	64.1 ± 4	98.7 ± 2	178.2 ± 3
4	102.7 ± 1	52.4 ± 1	50.3 ± 3	107.5 ± 1	156.3 ± 2
5	98.7 ± 2 <sup>b</sup>	46.2 ± 3	52.5 ± 3	85.7 ± 3 <sup>c</sup>	165.5 ± 2
6	110.2 ± 3	57.5 ± 2	52.7 ± 2	136.5 ± 2	149.4 ± 2

where a values are mean ± SEM : mg/dl

b  $P < 0.05$  vs. control

c  $p < 0.01$  vs. control

Table 5. The fraction of HDL and LDL, some factors ratios<sup>a</sup>

Group	HDL	LDL	TG/PL	F-CHOL/PL	T-CHOL/PL	HDL/T-CHOL
Control	35.8 ± 2	72.6 ± 2	0.60	0.27	0.62	0.33
1	49.9 ± 2 <sup>b</sup>	54.6 ± 3	1.84	0.39	0.54	0.48
2	48.5 ± 3 <sup>b</sup>	65.8 ± 2	0.68	0.45	0.68	0.42
3	50.6 ± 2 <sup>b</sup>	47.9 ± 3 <sup>c</sup>	0.55	0.19	0.55	0.51
4	46.4 ± 3 <sup>b</sup>	56.3 ± 4	1.04	0.34	0.69	0.45
5	49.4 ± 1 <sup>b</sup>	49.1 ± 3 <sup>c</sup>	0.52	0.28	0.50	0.50
6	43.9 ± 2 <sup>c</sup>	62.3 ± 2	0.91	0.39	0.74	0.40

Where a values are mean ± SEM : mg/dl

b  $p < 0.05$  vs. control

c  $p < 0.01$  vs. control

Table 6. Plasma protein fraction of rabbit<sup>a</sup>

Group	T-Protein	Albumin	Globulin				A/G
			$\alpha_1$	$\alpha_2$	$\beta$	$\gamma$	
Control	5.7 ± 2.0	4.19 ± 1.5	0.19 ± 1	0.45 ± 2	0.48 ± 1	0.39 ± 1	1.4
1	5.7 ± 1.5	3.42 ± 2.0 <sup>b</sup>	0.54 ± 2	0.42 ± 1	0.64 ± 1	0.68 ± 2	1.6
2	5.7 ± 1.5	3.74 ± 1.0	0.56 ± 2	0.60 ± 2	0.53 ± 1	0.83 ± 1	1.5
3	5.6 ± 2.0	3.93 ± 1.5	0.35 ± 1	0.46 ± 1	0.31 ± 1 <sup>c</sup>	0.55 ± 2	1.4
4	5.7 ± 1.5	3.95 ± 2.0	0.37 ± 2	0.65 ± 1	0.30 ± 2 <sup>b</sup>	0.53 ± 1	1.5
5	5.7 ± 2.0	3.64 ± 1.5 <sup>b</sup>	0.30 ± 2	0.59 ± 2	0.29 ± 1 <sup>c</sup>	0.68 ± 1	1.6
6	5.5 ± 2.0	3.98 ± 2.0	0.27 ± 2	0.35 ± 1	0.35 ± 1 <sup>b</sup>	0.55 ± 2	1.4

Where a values are mean ± SEM: mg/dl

b p < 0.05 vs. control

c p < 0.01 vs. control

ginseng powder plus magnesium, fed korea ginseng powder plus magnesium and perilla oil had a significant decrease compared to the control group.

The phospholipid in the plasma was lower level than the control diet group, but the animals fed korea ginseng powder showed a little bit decreased compared to the control group.

LDL fractions were decreased on the animals were fed perilla oil plus magnesium, and magnesium plus perilla oil and korea ginseng powder. It showed a significant difference compared to the control group (P < 0.05). HDL fraction was significantly increased in rabbits fed the magnesium plus perilla oil, calcium plus perilla oil, magnesium plus perilla oil and korea ginseng powder (P < 0.05), and calcium plus perilla oil and korea ginseng powder (P < 0.01). HDL/T-CHOL ratio has been suggested as an indicator of prevental possibility in arteriosclerosis, the values would be increased to 1.0, then the plasma cholesterol concentration could be decreased.

## 2. Plasma protein fraction

The plasma protein fraction response to the different dietary regimens show Table 6.

The animals fed with magnesium and calcium added diet shows lower than the control groups. The animals fed with korea ginseng powder and basal diet shows the lowest concentration in magnesium added groups. Albumins were decreased on the

Table 7. Plasma electrolytes concentrations<sup>a</sup>

Group	(mg %)		
	Na <sup>+</sup>	K <sup>+</sup>	Na/k
Control	322 ± 1.5	19.5 ± 1.0	16.5
1	338 ± 1.5	16.4 ± 2.0	20.6
2	329 ± 1.5	16.7 ± 1.5	19.7
3	322 ± 2.0	16.4 ± 2.0	19.6
4	334 ± 1.5	17.1 ± 2.0	19.5
5	320 ± 1.5	18.6 ± 1.5	17.2
6	327 ± 2.0	17.1 ± 1.5	19.1

a Values are mean ± SEM

animals were fed magnesium plus korea ginseng powder, and magnesium plus perilla oil and korea ginseng powder compared to the control diet group. Beta-globulin was significantly decreased in rabbits fed perilla oil plus magnesium and perilla oil plus magnesium and korea ginseng, (P < 0.01), fed perilla oil plus calcium, and perilla oil plus calcium and korea ginseng powder (P < 0.05), compared to the control.

## 3. Plasma electrolytes

Plasma electrolytes (sodium and potassium ion) was analyzed by the fluorometry. The results showed Table 7.

In the electrolytes, the concentration of sodium ion was decreased in the rabbits fed magnesium plus perilla oil and korea ginseng powder compared to the control group, but there was no significant differ-

ences. The concentration of potassium ion was decreased all the animals compared to the control group. In general, as the concentration of sodium ion shows higher than 328mg%, it suggest that the animals is nepritis and hypercorticoadrenalism. As the concentration of potassium ion shows lower than 17.6mg%, it suggest that the animals are hyperinsulinism diabetts and hypercorticoadrenalism. Therefore, ginseng powder would be acted on hormonal active.

#### 4. Plasma calcium and magnesium concentration

The concentration of calcium and magnesium in rabbit plasma was determined by the EDTA-chelometry. The results show table 8.

The concentration of calcium and magnesium in the rabbits does not show a great differences in the all experimental groups animal fed korea ginseng powder. In general calcium to magnesium ion concentration showed one to one and half or two times.

#### 5. Microsomal cholesterol and phospholipd content

Microsomes prepared from livers of animals on the dietary regimens were analyzed for cholesterol and phospholipid content. Hepatic microsomal total cholesterol was significantly decreased in rabbits fed magnesium + perilla oil and magnesium + perilla oil + korea ginseng powder, compared to microsomes obtained from animals fed the control diet. The results show table 9.

It is known that the ratio of total cholesterol to phospholipid in plasma and microsomas is 0.62, 0.54, 0.68, 0.55, 0.69, 0.50 and 0.74 in plasma, respectively and 0.059, 0.051, 0.049, 0.041, 0.048 and 0.041 in microsomes, respectively.

#### 6. Microsomal HMG-CoA reductase activity

The effects of the diets on HMG-CoA reductase activities show Table 10.

Through the Table 10, hepatic HMG-CoA reductase activity was increased a little bit by dietary korea ginseng powder and magnesium and calcium, compared to the control diet group. Total activity of

Table 8. The concentration of Ca<sup>2+</sup> and Mg<sup>2+</sup> in plasma<sup>a</sup>

Group	Ca <sup>2+</sup>	Ma <sup>2+</sup>	Ca/Mg
Control	10.4 ± 1.5	6.5 ± 1.5	1.6
1	10.5 ± 2.0	6.9 ± 1.5	1.5
2	11.2 ± 2.0	6.7 ± 2.0	1.7
3	10.9 ± 1.5	7.8 ± 1.5	1.4
4	11.6 ± 2.0	8.0 ± 1.5	1.5
5	9.8 ± 2.0	6.5 ± 2.0	1.5
6	10.8 ± 1.5	6.8 ± 1.5	1.6

<sup>a</sup> Valuves are mean ± SEM

Table 9. Effect of diet on microsomal cholesterol and phospholipids content in liver<sup>a</sup>

Group	T-Cholesterol	Phospholipid	T-CHOL/PL
Control	25.5 ± 1.5	425.6 ± 2.0	0.059
1	23.4 ± 1.5	467.5 ± 1.5	0.051
2	25.7 ± 2.0	482.1 ± 2.0	0.049
3	20.7 ± 1.5 <sup>b</sup>	495.7 ± 1.5 <sup>c</sup>	0.041
4	24.5 ± 1.5	502.4 ± 2.0 <sup>c</sup>	0.048
5	21.7 ± 2.0 <sup>b</sup>	452.4 ± 2.0	0.048
6	29.4 ± 1.5	475.2 ± 1.5	0.041

<sup>a</sup> values are mean ± SEM, µg/mg

<sup>b</sup> p < 0.05 vs. control

<sup>c</sup> p < 0.01 vs. control

HMG-CoA reductase in animal fed magnesium + korea ginseng powder and magnesium + perilla oil + korea ginseng powder were higher than the control diet group. Total activity of HMG-CoA reductase in animal fed calcium + korea ginseng powder and calcium + perilla oil + korea ginseng poder was lower than the animals fed magnesium, perilla oil and korea ginseng powder.

## IV. DISCUSSION

It is of interest that, in the rabbit, the ingestion of perilla oil plus magnesium, perilla oil plus magnesium and korea ginseng powder, resulted in a decreased in plasma cholesterol, compared to the control diet group. In the perilla oil, the w-3(30:3) polyunsa-

Table 10. Effect of diets on HMG-CoA reductase activity in rabbit liver

Group	Total activity	Specific activity	Microsomal protein
control	295.34 ± 1.5	16.33 ± 2.0	18.1 ± 1.0
1	300.11 ± 2.0	16.67 ± 1.5	18.0 ± 2.0
2	296.67 ± 1.5	16.67 ± 2.0	17.8 ± 1.5
3	309.33 ± 1.5	16.99 ± 1.0	18.2 ± 1.5
4	300.71 ± 1.0	16.34 ± 1.5	18.4 ± 2.0
5	307.33 ± 1.0	17.66 ± 1.5	17.4 ± 1.5
6	300.67 ± 2.0	16.34 ± 2.0	18.4 ± 1.5

A Values are mean ± SEM.

turated fatty acid is 58.3%, and *w*-6(18:2) polyunsaturated fatty acid is 15.6%, *w*-6/*w*-3 ratio is 0.27 and *p/s* ratio is 9.8. In the rabbit animals, plant oil which is enriched in *w*-6 polyunsaturated fatty acid such as safflower oil, increased plasma cholesterol, Field, et al. (31-34). But the animals rabbit fed menhaden oil, *w*-3 polyunsaturated fatty acid is 27% and cocoa butter *w*-3 is 1.0% showed 106mg% in menhaden oil and 79mg% in cocoa butter, Marthur et al. (46)

The regulation of hepatic HMG-CoA reductase activities by the different dietary regimens was studied. In the liver, the activity of HMG-CoA reductase was increased a little bit in hepatic microsome prepared from rabbits ingesting the perilla oil and magnesium plus korea ginseng powder. In the earlier study, hepatic HMG-CoA reductase activity was less in rats fed a diet rich in polyunsaturated fatty acids compared to the activity observed in rats fed a diet rich in long-chain saturated fatty acids, Okamatsu et al (31) and Mitropoulos et al (47).

They mentioned that the HMG-CoA reductase is regulated by the degree of polyunsaturated and not by any differences in the class of polyunsaturated fatty acids. But Rodgers et al (48) mentioned that there was no effect of dietary fat saturation on HMG-CoA reductase activity. They suggested that the ratio of *w*-6/*w*-3 is an important factor for HMG-CoA reductase activity increased or not.

Several investigators have reported that much intake of carbohydrates were associated with lower

levels of high density lipoprotein cholesterol (49-50). Therefore, it is expected that much more consumption of sucrose to correlate with lower HDL cholesterol levels. Some investigators mentioned that intake of greatly increased amounts of polyunsaturated fatty acids resulted in decreased in HDL cholesterol (50). But Ernst et al (51) mentioned that there was no consistent relation of dietary cholesterol and polyunsaturated or saturated fatty acids intakes with levels of HDL cholesterol.

High serum and plasma cholesterol levels represent one of the major risk factor for arteriosclerosis and hypertension. While a major portion of the serum cholesterol exists as a component of low density lipoproteins in men. Recently suggested that low concentration of serum high density lipoproteins are associated with higher rates of coronary heart disease (52-53). HDL cholesterol is inversely correlated with the incidence of coronary artery disease (53-54).

In the previous study (55-58), perilla oil and magnesium showed plasma cholesterol level lowering effect. Through this experiments, magnesium, perilla oil and korea ginseng powder would be effected as a factor of decreasing of serum and plasma cholesterol concentration, increasing of HMG-CoA reductase activity, increasing of phospholipids concentration, normal state of calcium and magnesium metabolism. Of increasing of sodium ion and decreasing of potassium ion concentration.

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