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Effects of Garlic Products on Lipid Metabolism in Cholesterol-Fed Rats[1] (41494)

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Abstract. The effect of garlic prepared in several forms on lipid metabolism was studied in male rats fed a diet containing 1% cholesterol. Garlic was supplemented at 2% of the diet as fresh garlic in forms of ethanol extracted garlic residue, ethanol extract of garlic, whole garlic, and autoclaved garlic. Diets were fed for 4 weeks from 6 weeks of age. The supplementation of garlic products except ethanol-extracted garlic residue reduced plasma and liver cholesterol levels. The reduction in the plasma cholesterol by feeding garlic products was in very low density lipoprotein and low-density lipoprotein cholesterol fractions. Animals fed diets supplemented with garlic decreased liver glucose-6-phosphate dehydrogenase and malic enzyme activities and also reduced the liver weight, inquinal adipose tissue weight, liver total lipids, and plasma triglycerides. The hypocholesterolemic activity of garlic was contained in the ethanol extract and stable when autoclaved at 120° for 1 hr.

The blood cholesterol level has been reported as an independent risk factor contributing to the development of coronary heart diseases (1–3). Recent studies have shown that essential oil from garlic or onion reduces the blood cholesterol level in humans (4, 5) and animals (6, 7). The essential oil of garlic or garlic extract prevented lipid accumulation in the aorta and showed preventive effects against pathogenic atherosclerosis in rabbits fed an atherogenic diet (6, 7). An oral administration of garlic to human subjects depressed platelet aggregation (5, 8) and the blood glucose concentration (9). Garlic is used as a flavoring agent world wide. Garlic also has been known to have medicinal properties in oriental countries. Lyophilized garlic powder added at 2% level to a diet containing 1% cholesterol decreased the cholesterol and lipid levels in the blood and liver by increasing fecal excretion of neutral and acidic sterols in rats (10).

Garlic can be processed into different products and used. The objective of this study was to determine the effects of selected garlic products on lipid metabolism in rats including the determination of each lipoprotein cholesterol fraction and hepatic lipogenic enzyme activities.

Materials and Methods. Male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were individually housed in stainless-steel cages in a room maintained at 22°–24° with about 50% relative humidity. The room was lighted from 06:00 to 18:00 hr. The composition of a basal diet (control group) containing 1% cholesterol is given in Table I. Garlic bulbs were obtained from a commercial source, peeled off, washed and ground by a blender for 2 min with an addition of water. A portion of ground garlic was lyophilized and used as whole garlic. The moisture contents of the fresh garlic bulb and dried garlic powder were 69.1 and 1.1%, respectively. Another portion of ground garlic was refluxed with 50% aqueous ethanol (v/v) in a 60° water bath for 2 hr, filtered, and ethanol was evaporated with N gas flow. The resulting solution was concentrated by lyophilization and used as garlic extract (22.1% of whole fresh garlic). The filtered garlic particles (8.9% of whole fresh garlic) were lyophilized and used as garlic residue. A portion of ground garlic was autoclaved for 1 hr at 120°, lyophilized and used as autoclaved garlic.

There were five dietary treatments: (1)

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TABLE I. COMPOSITION OF THE BASAL DIET

Ingredient	g/100 g diet
Casein[a]	20.00
Dextrose[a]	25.00
Corn starch[a]	40.35
Corn oil[b]	5.00
Cellulose[c]	2.50
Mineral mix[d,e]	4.00
Vitamin mix[f,g]	2.00
DL-Methionine[a]	0.15
Cholesterol[h]	1.00

[a] ICN Pharmaceuticals, Inc., Cleveland, Ohio.

[b] Tocopherol stripped, ICN Pharmaceuticals, Inc.

[c] Brown and Co., Boston, Mass.

[d] Supplied by J. T. Baker Chemical Co., Phillipsburg, N.J.

[e] Provided per kilogram diet; $CaCO_3$, 7.5 g; $CaHPO_4 \cdot 2H_2O$, 8.2 g; $CuSO_4 \cdot 5H_2O$, 0.023 g; $Fe(C_6H_{11}O_7)_2$, 0.9 g; KIO_3, 0.01 g; K_2HPO_4, 13.0 g; $MgSO_4 \cdot 7H_2O$, 4.8 g; $MnCO_3$, 0.2 g; NaCl, 4.5 g; Zn CO_3, 0.052 g.

[f] Vitamins except tocopherol, retinyl palmitate and cholecalciferol supplied by General Biochemicals, Chagrin Falls, Ohio; tocopherol, retinyl palmitate, cholecalciferol supplied by Hoffman-LaRoche, Nutley, N.J.

[g] Provided per kilogram diet; thiamin·HCl, 10 mg; riboflavin, 10 mg; nicotinic acid, 40 mg; calcium pantothenate, 30 mg; folic acid, 3 mg; inositol, 25 mg; biotin, 0.2 mg; vitamin B-12, 0.02 mg; menadione, 2 mg; retinyl palmitate, 10,000 IU; cholecalciferol, 2000 IU; DL-α-tocopheryl acetate, 120 IU; choline chloride, 1500 mg.

[h] Eastman Kodak Co., Rochester, N.Y.

basal diet (control), (2) control plus garlic residue, (3) control plus garlic extract, (4) control plus whole garlic, (5) control plus autoclaved garlic. Garlic products were added to the diet equivalent to 2% of a diet as fresh garlic (w/w) at the expense of corn starch. Rats were fed a stock diet for the 2-week preexperimental period. At 6 weeks of age, experimental diets and water were provided *ad libitum* for the next 4 weeks. Each dietary treatment was randomly assigned to 10 individually caged rats. The body weight and food consumption were determined weekly.

Chemical assay. Blood samples were taken into heparinized Vacutainer (Beckton-Dickinson, Dickinson and Co., Rutherford, N.J.) tubes by cardiac puncture; the rats were in a weakly anesthetized state following phenobarbital administration (after fasting for 16 hr). Immediately after the blood sampling the liver was excised, washed in chilled saline solution, blotted and cooled in crushed ice. A part of liver was used for enzyme assay and the rest of the liver was kept at −35°C until analysis of lipid components. The inquinal fat pads in both sides were removed at around caput epididymis and weighed.

Plasma triglycerides were analyzed enzymatically using Tri-Es (Harleco, Division of American Hospital Supply Co., Gibson, N.J.). Plasma lipoprotein cholesterol was separated by ultracentrifugation (11) and plasma total and each lipoprotein cholesterol fraction was determined using Beckman Cholesterol Analyzer-2 and enzymatic cholesterol assay kit (Beckman Instruments, Inc., Fullerton, Calif.). Liver lipids were determined gravimetrically after extraction according to the method of Folch *et al.* (12) and liver cholesterol was analyzed by the method of Kim and Goldberg (13).

Enzyme assay. Approximately 1.0 g of liver was homogenized in 9 ml of 0.15 *M* KCl and centrifuged at 20,000*g* for 1 hr. The resulting supernatant was used for determining enzyme activity and protein. Glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49) was assayed by the method of Kornberg and Horecker (14) and malic enzyme (ME) (EC 1.1.1.40) was assayed by the method of Ochoa (15). The reaction was initiated by adding the enzyme source to the medium. The assay was conducted at 30° by observing changes in absorbancy at 340 nm with a Beckman Model-25 Spectrophotometer (Beckman Instruments, Inc.). The protein content of the supernatant fraction was determined by the method of Lowry *et al.* (16). Enzyme activity was expressed as units per milligram protein where a unit was the amount of enzyme which converted one nanomole of substrate per minute at 30°.

Statistical analysis. The data were analyzed statistically using the analysis of variance technique and the least significant difference procedure was used in comparisons of treatment means (17).

Results. Table II and Fig. 1 summarize the results of the experiment. Rats fed the diet supplemented with garlic extract consumed less food than the control and whole

TABLE II. EFFECTS OF GARLIC PRODUCTS ON THE WEIGHT GAIN, FOOD CONSUMPTION, AND PLASMA AND LIVER COMPONENTS IN MALE RATS[a]

	Dietary treatments				
	Control	Garlic residue	Garlic extract	Whole garlic	Autoclaved garlic[b]
Weight gain, g/day	7.5 ± 0.5 A	7.2 ± 0.5 A,B	6.7 ± 0.3 B	6.4 ± 0.3 B	6.6 ± 0.4 B
Food intake, g/day	18.2 ± 1.4 A	17.1 ± 2.0 A,B	16.2 ± 1.4 B	17.2 ± 2.2 B	17.5 ± 1.2 B
Plasma triglycerides, mg/dl	133.4 ± 20.6 A	122.1 ± 16.7 A	91.4 ± 12.9 B	96.4 ± 13.0 B	97.3 ± 18.1 B
Plasma total cholesterol, mg/dl	102.9 ± 11.8 A	97.7 ± 17.6 A	74.6 ± 8.3 B	77.6 ± 7.8 B	78.3 ± 14.6 B
Plasma free cholesterol, mg/dl	29.1 ± 4.2 A	27.5 ± 3.1 A,B	24.6 ± 2.0 B	24.2 ± 2.6 B	24.0 ± 3.2 B
Liver weight[c]	5.07 ± 0.68 A	4.56 ± 0.45 A,B	3.93 ± 0.54 B	3.96 ± 0.35 B	4.11 ± 0.48 B
Inquinal fat pad weight[d]	1.11 ± 0.28 A	1.06 ± 0.16 A,B	0.85 ± 0.16 B	0.87 ± 0.20 B	0.89 ± 0.14 B
Liver total lipid, mg/g liver	72.1 ± 11.5 A	69.4 ± 13.8 A	41.4 ± 9.2 B	41.1 ± 7.0 B	52.6 ± 10.6 B
Liver total cholesterol, mg/g liver	15.88 ± 1.94 A	14.76 ± 2.47 A	6.33 ± 1.06 B,C	5.23 ± 0.84 C	8.83 ± 1.63 C
Liver free cholesterol, mg/g liver	4.88 ± 1.07 A	3.97 ± 0.80 A,B	2.33 ± 0.36 B	2.56 ± 0.29 B	3.38 ± 0.68 B
Liver G-6-PDH,[e] unit/mg protein	9.83 ± 1.36 A	9.20 ± 1.06 A	6.13 ± 1.34 B	6.97 ± 1.37 B	6.40 ± 1.74 B
Liver ME,[e] unit/mg protein	5.73 ± 1.03 A	5.82 ± 1.76 A	3.53 ± 0.82 B	3.69 ± 0.93 B	3.68 ± 0.68 B

[a] Mean ± standard deviation of 10 rats. Means bearing different letters at the same row are significantly different ($P < 0.05$).
[b] Mean ± standard deviation of 9 rats.
[c] Liver weight in g/100 g body weight.
[d] Two inquinal fat pad weights in g/100 g body weight.
[e] G-6-PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme.

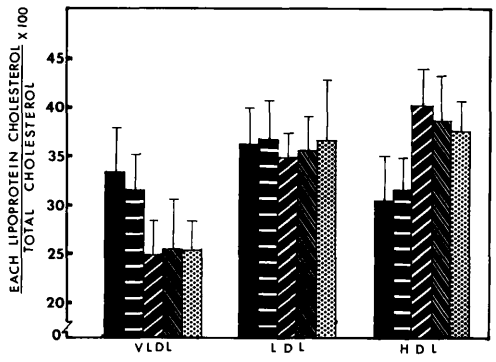

FIG. 1. Effects of garlic products on plasma very low density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol as a percentage of the plasma total cholesterol. ▬, Control; ▮▮▮, garlic residue; ▨, garlic extract; ▨, whole garlic; ▨, autoclaved garlic. Each bar indicates mean ± standard deviation of 10 rats except the autoclaved garlic group which is mean ± standard deviation of 9 rats.

garlic groups. Body weight gain of rats fed garlic products except garlic residue was decreased as compared with rats fed the control diet (Table II). The plasma concentrations of triglycerides, total cholesterol, and free cholesterol were decreased by the supplementation of garlic extract, whole garlic, or autoclaved garlic.

The plasma cholesterol was fractionated into each lipoprotein cholesterol and the percentage of each lipoprotein cholesterol to the plasma total cholesterol is given in Fig. 1. The proportion of very low density lipoprotein (VLDL) cholesterol to the total plasma cholesterol was significantly ($P < 0.05$) lower in rats fed the garlic extract, whole garlic, or autoclaved garlic diets than in animals fed the control diet. Just opposite results were obtained in the proportion of high-density lipoprotein (HDL) cholesterol to the plasma total cholesterol.

The supplementation of garlic extract, whole garlic, or autoclaved garlic also reduced the liver weight, inquinal fat pad weights, liver total lipid, and liver total and free cholesterol (Table II). Rats fed the diets added with garlic products except garlic residue decreased hepatic G-6-PDH and ME activities as compared with control animals.

Discussion. Garlic appeared as an effective agent to reduce blood cholesterol and triglycerides as well as liver lipids and cholesterol. The reduction in plasma cholesterol was in VLDL and LDL cholesterol as observed in a previous study (10). The VLDL cholesterol values (mean ± SD) for control, garlic residue, garlic extract, whole garlic, and autoclaved garlic groups were 34.3 ± 5.0, 31.0 ± 2.9, 18.4 ± 2.4, 20.0 ± 2.7, and 20.1 ± 2.6 mg/100 ml, respectively, and LDL cholesterol values (mean ± SD) for the same treatments were 37.3 ± 3.6, 35.5 ± 4.2, 26.1 ± 2.2, 27.8 ± 2.9, and 28.5 ± 3.7 mg/100 ml, respectively. Although the actual values of HDL cholesterol among treatment groups (31.2 ± 4.1, 31.2 ± 3.0, 30.1 ± 2.8, 30.0 ± 4.2, and 30.6 ± 2.8 mg/100 ml for control, garlic residue, garlic extract, whole garlic, and autoclaved garlic, respectively) were similar, the percentage of HDL cholesterol to the plasma total cholesterol was higher in animals fed the garlic products except garlic residue than the control group. This was because the plasma total cholesterol level was much lower in those garlic groups than in the control group. The importance of plasma cholesterol and lipoprotein concentrations in the pathogenesis of atherosclerosis has been noted by numerous investigators. Increased levels of total plasma cholesterol with increases in LDL or VLDL were associated with a greater risk of developing coronary heart diseases (1, 2) while high concentration of HDL appeared to be protective (18, 19). The changes in the proportions of lipoprotein cholesterol fractions may be one reason of protective effect of garlic against the atherosclerotic process.

G-6-PDH and ME activities have been shown to be correlated to lipogenic capacity in various tissues including rat liver (20) by supplying substrates for fatty acid synthesis (21). Garlic appeared to reduce fatty acid synthesis by decreasing key enzyme activities in supplying substrates and consequently reduced the lipid levels in the liver and plasma. The decrease in food intake in some of garlic-fed rats did not cause these metabolic changes since pair-fed animals with and without garlic in a previous

study (10) showed similar results as in the present study. The lesser weight gain in rats fed garlic products might be the result in part of decreases in food intake and in part of the metabolic changes due to garlic feeding. The observed metabolic changes in garlic-fed animals were not likely due to hepatic damage: the liver and gastrointestinal tract of rats were grossly examined for any tissue damage and found no visible tissue damage in controls and garlic groups.

Garlic was similarly effective in lowering cholesterol and lipid levels in the plasma as well as in the liver in a form of lyophilized whole garlic or ethanol extract of garlic suggesting that the active agent in garlic for reducing blood cholesterol was contained in the garlic extract. The nonsignificance of garlic residue, the portion after ethanol extract of whole garlic, for hypocholesterolemic activity also confirms the effectiveness of ethanol extract of garlic. Autoclaving the garlic at 120° for 1 hr did not diminish the hypocholesterolemic property of garlic.

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