EFFECTS OF GARLIC OIL ON SERUM AND TISSUE LIPIDS OF RATS FED DIETS WITH TWO LEVELS OF BEEF TALLOW

by

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INTRODUCTION

Although the United States has experienced a 4.7% decline in the coronary heart disease (CHD) mortality rate from 675,000 deaths in 1968 to 643,000 deaths in 1975, this mortality rate still ranks among the highest in the world (1). CHD also is a major cause of death in other industrialized nations of the world.

Extensive research continues to expose the dominant and complex role of nutrition in CHD but the interactions between numerous factors recognized as contributory to CHD are not yet clear. Epidemiological data have established hypercholesterolemia as a major risk factor in atherosclerosis, although a causal relationship has not been proved. Numerous international studies have shown that populations differing in habitual intake of saturated fat and cholesterol also differ markedly in serum cholesterol and CHD incidence rates (2,3,4). One of the most comprehensive prospective international studies on CHD is the International Cooperative Study of the Epidemiology of Cardiovascular Disease or the Seven Country Study (5). Observations of approximately 12,000 men aged 40-59 years were taken from 18 population groups in seven countries: Finland, Greece, Italy, Japan, the Netherlands, the United States and Yugoslavia. Significant positive correlations were found between saturated fat intake and CHD incidence rate, saturated fat intake and serum cholesterol level and serum cholesterol level and CHD.

Other risk factors such as cigarette smoking, obesity, hypertension, increasing levels of serum lipids and electrocardiograph abnormalities have been associated with CHD (6), but serum cholesterol remains the single best

indicator of risk of premature atherosclerotic disease, including CHD (7). Prevention and management of CHD often has been directed at normalizing elevated serum cholesterol levels either through dietary intervention (8,9,10) or through the use of lipid-lowering drugs (11, 12) although no one treatment has been established as the "method of choice."

A decrease in serum cholesterol has traditionally been achieved by several different mechanisms: inhibition of cholesterol biosynthesis, inhibition of cholesterol absorption, increased conversion of cholesterol to bile acids, and increased fecal excretion of cholesterol (13). Recently, Sodhi et al. (14) suggested that hypocholesterolemics should be directed toward altering the metabolism of various plasma lipoproteins rather than non-specifically lowering total serum cholesterol values.

Although most hypolipemic agents are pharmacological in origin, several natural substances such as ginger (15), curcumin, the coloring agent in turmeric (16), onion and garlic (17) have shown hypocholesterolemic effects in clinical experiments. Folk medicine has attributed numerous preventive or curative properties to garlic (18, 19), but the question remains whether all of these claims can be justified by scientific research. Garlic has been shown to affect several blood coagulation factors which may play an important role in what is often the final event in CHD, coronary thrombosis. In several studies, garlic inhibited platelet aggregation and enhanced fibrinolytic activity (20) and decreased serum fibrinogin and whole blood coagulation time (21).

Numerous studies also have shown a number of mechanisms by which garlic may offer protection against factors associated with CHD. Specifically, in animal models with experimentally induced atherosclerosis, garlic has been

shown to decrease: serum and tissue cholesterol, serum triglycerides and phospholipids, free cholesterol to ester cholesterol ratio, severity of atherosclerotic lesions and the ratio of β to α lipoproteins (17, 22). However, the effect dietary fats may have on the action of garlic has not been investigated. The activity of another hypolipemic agent, pectin, has been shown to be altered both by the amount and type of dietary fat (23). Freshly extracted garlic juice or oil rather than commercially available garlic oil has been used in previous studies.

The objective of this study was to determine the effects of commercial garlic oil on serum and tissue lipid levels in male weanling rats fed cholesterol supplemented diets with low and high levels of beef tallow.

REVIEW OF LITERATURE

Botanical Information

Garlic (Allium sativum), onion (Allium cepa) and chives (Allium schoenoprasum) are members of the Amaryllidaceae or lily family. The name garlic is derived from the Anglo-Saxon garleac (gar = spear, leac = plant), meaning spear plant (24). Garlic and onion are among the oldest cultivated crops. Garlic bulbs were found in the tomb of Tutankhamen, 1358 B.C. and inscriptions on other tombs recorded exact amounts of garlic consumed by Egyptian laborers(18).

Garlic is a hardy perennial with long, solid, flattened leaves. The edible bulb is composed of egg-shaped, pungent segments called cloves and is encased in a whitish membrane. A stalk rising directly from the bulb produces lavender or whitish flowers which drop prior to harvest (24).

Garlic is favored by a sunny climate, adequate weed control, irrigation and a sandy loam soil. From each pound of garlic planted, approximately 5-7 lbs are harvested, or about 7,000-10,000 lbs per acre. The United States, Bulgaria, Hungary and Taiwan produce the major garlic crops in the world (24). Gilroy, California is considered the "garlic capitol of the world" and hosts an annual garlic festival.

Chemical composition

Investigation into the chemical composition of garlic began as early as 1892 when Semmler subjected garlic to steam distillation and produced a brownish-yellow, obnoxious smelling oil with a yield of 0.1-0.2% (19). Further fractional distillation identified several organic compounds in garlic oil: principally, diallyl disulfide, $C_6H_{10}S_2$, along with diallyl

trisulfide, diallyl polysulfide and a small amount of diethyl disulfide (18,19). Other sources (25, 26) list garlic oil in concentrations of 0.06-0.1% and mention allyl propyl disulfide, $C_6H_{12}S_2$ as another constituent. Onion is believed to contain similar sulfur-containing compounds but in smaller concentrations of about 0.005% (27).

The Food Chemicals Codex (FCC) (28) lists the following specifications of garlic oil: refractive index of 1.559-1.579 and specific gravity between 1.040 and 1.090. The oil is described as a reddish-orange liquid with a strong odor which is soluble in most fixed oils and mineral oil and may be incompletely soluble in alcohol. The oil is insoluble in glycerin and in propylene glycol.

The characteristic, pungent odor of garlic is not present in the whole garlic bulb until it is crushed, suggesting the odorous compound(s) associated with garlic is formed through enzymatic cleavage of a precursor (19). Investigation of the precursor, S-allyl cysteine sulfoxide, and the odorous compound, diallyl thiosulfinate, $(C_3H_5S)_2O$, provided the scheme shown in Figure 1 (19, 29). Another sulfur-containing amino acid, S-methyl-L-cysteine, has been identified from garlic extracts (30).

The S-allyl cysteine sulfoxide content of garlic runs approximately parallel to its sulfur content. Table 1 shows the variation in sulfur content of garlic obtained from several sources (19). S-allyl cysteine sulfoxide is relatively odorless, but upon enzymatic cleavage by alliinase, the compound, diallyl thiosulfinate, that is produced is about 2.5% soluble in water, is relatively unstable and carries the typical but not unpleasant odor of garlic (31). Prior to 1945, diallyl thiosulfinate was called allicin and S-allyl cysteine sulfoxide was called alliin (32). The term allicin was

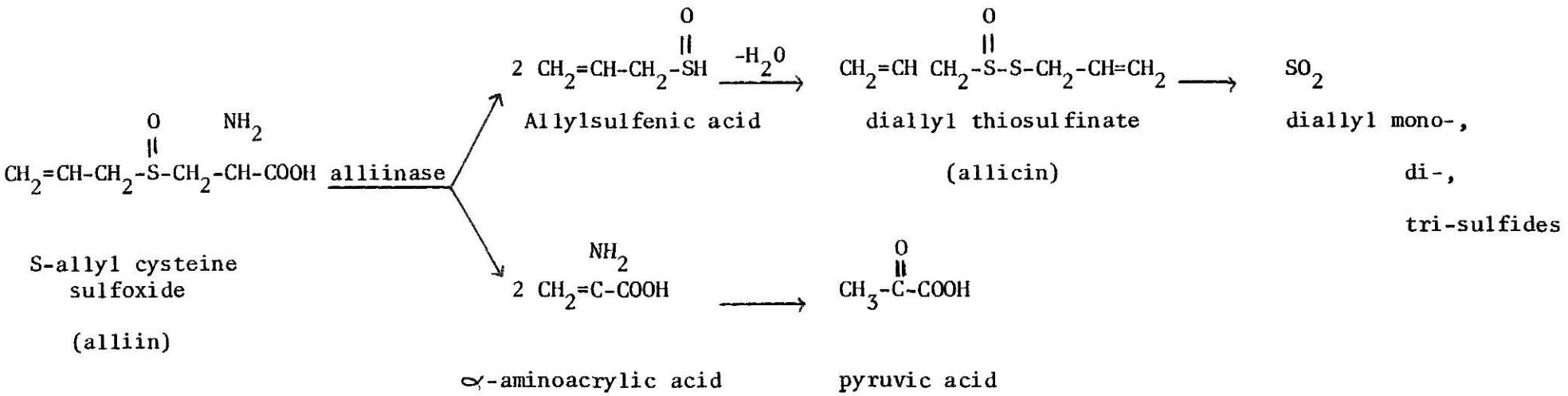

Fig. 1. Decomposition of S-allyl cysteine sulfoxide (19,33).

TABLE 1

Sulfur content of garlic of various origin (19)

Place	sulfur
	mg/kg
Basel, experimental garden	3720
Basel	1100
Reinach near Basel	550
Reihen (Basel)	410
Tecknau	1400
Muntschemeir	1023
Finsterhennen	510
Muri (Aargau)	1675
Banolas (Spain)	1150
Villafranca (Spain)	970
Navarra (Spain)	1220
Piacenza (Italy)	1790

TABLE 2

Components of garlic extract[1] (33)

Component	
	%
Allyl alcohol	5.4
Methyl allyl disulfide	1.2
Diallyl disulfide	5.7
Dimethyl trisulfide	2.4
Methyl allyl trisulfide	1.5
(144-I)[2]	23.5
Diallyl trisulfide	1.0
(144-II)[2]	55.4

[1]As determined by gas-liquid chromatography (GLC) of synthetic diallyl thiosulfinate on columns coated with SCOT carbowax programmed at 4°C per minute, from 70-170°C.

[2]Products of diallyl thiosulfinate decomposition during GLC, not components of garlic.

dropped because of possible association with pharmacological agents with similar names.

According to Brodnitz et al. (33), diallyl thiosulfinate undergoes a nonenzymatic rearrangement upon steam distillation or at room temperature for 24 hours to form sulfur dioxide, diallyl mono-, di-, and tri-sulfides. These products are considered the major components of commercial garlic oil, the odor of which is described as intensely obnoxious. Table 2 shows the relative concentrations of these sulfur containing compounds in commercial garlic oil (33).

Consumption data and nutritive value

Per capita consumption of fresh garlic in the United States, as estimated by the U.S. Department of Agriculture, has risen steadily from 0.2 lbs per year in 1920 (34) to 0.6 lbs in 1975 and 0.5 lbs in 1977 (35). Garlic also is available in many processed forms; powdered, instant granulated, instant ground, instant minced, chopped, sliced, and garlic salt. The U.S. per capita consumption data mentioned previously includes only the raw form of garlic and does not account for the nearly 70% of the garlic crop that is converted to dehydrated forms (36). According to Rosengarten (24), the consumption of garlic, particularly in the dehydrated forms, has increased 1,000% in the U.S.

Onions, which may possess similar properties as garlic, are consumed in the U.S. in greater quantities than garlic and per capita consumption has shown little variation since 1920. Yearly per capita consumption of onions and shallots was estimated at 12.2 lbs in 1920 (34) and 12.3 lbs in 1977 (35).

The Food and Agricultural Organization (FAO) of the United Nations' provisional food balance sheets show the wide variation in garlic consumption

among countries (Table 3). Argentina, Egypt, Spain, and Yugoslavia are countries where per caput supply of garlic is particularly high. The per caput supply of garlic and onion in the United States is estimated to be 0.6 g/day and 16.7 g/day, respectively. Table 4 gives the nutritive value for both garlic and onion (38). The small amount of garlic and onion consumed by an individual would contribute little to his nutrient intake.

Anti-thrombotic effects of garlic

Investigation into the role of the thrombotic process in atherosclerosis has become an emerging field in CHD research. The precipitating factor in myocardial infarction (MI) is widely thought to be caused by thrombis in a coronary artery. The fact that both dietary factors (39, 40) and pharmacological agents (41) can alter the tendency to thrombis has provided another pathway by which CHD may be prevented or controlled.

Fibrinolytic activity (FA). Garlic has been shown to be a potent agent in increasing the FA in both animals (17, 42) and humans (43). Bordia et al. (17) observed that cholesterol feeding in rabbits decreased ($P \leq 0.001$) the FA and supplementation with garlic or onion oil increased ($P \leq 0.001$) the FA of plasma even above the normal levels of control animals receiving no cholesterol. In a three week study in which 6 male human subjects ingested 5.0 g of crushed garlic/day, FA of blood increased during the second ($P \leq 0.05$) and again during the third week ($P \leq 0.01$) (43). Garlic also has been reported to have a significant protective action against the decrease in FA normally observed in fat-induced alimentary lipemia (44).

Bordia et al. (45) investigated the effects of garlic oil (equivalent to 1 g raw garlic/kg) on FA of patients with a history of MI. The actual

TABLE 3

FAO estimate of per caput supply of garlic and onions[1] (37)

Country	Garlic		Onions, dry Onions & shallots, green	
	kg/year	g/day	kg/year	g/day
United States	0.2	0.6	6.1	16.7
Argentina	2.1	5.6	10.5	28.7
Brazil	0.5	1.3	3.0	8.1
Bulgaria	1.3	3.7	11.6	31.8
Burma	0.7	1.9	3.9	10.6
Chile	0.8	2.1	4.5	12.4
Czechoslovakia	0.6	1.7	8.7	23.8
Dominican Republic	1.0	2.8	4.0	10.9
Egypt	4.2	11.6	12.3	33.8
Finland	--	--	1.7	4.7
France	0.6	1.7	4.2	11.4
Germany, Fed. Rep. of	--	0.1	3.9	10.6
India	0.4	1.1	2.4	6.4
Israel	0.8	2.3	5.0	13.6
Italy	0.9	2.4	6.6	18.0
Japan	--	0.1	14.3	39.2
Korea, Rep.	2.3	6.4	4.5	12.4
Netherlands, Antilles	0.2	0.5	--	--
Pakistan	0.4	1.0	3.1	8.5
Paraguay	0.7	2.0	7.6	20.8
Spain	3.8	10.5	19.9	54.4
Sweden	--	--	2.8	7.6
Switzerland	0.1	0.3	2.8	7.8
Syria	1.4	3.8	14.2	39.0
Thailand	0.9	2.4	1.2	3.4
Turkey	1.0	2.6	13.5	36.9
Yugoslavia	2.3	6.2	11.2	30.6

[1]Per caput supply = total supplies available for human consumption/total population consuming food.

TABLE 4

Nutritive value of raw garlic and onion (38)

Nutrient	Garlic (cloves, raw)	Onion (mature, dry, raw)
	100 g	100 g
Water, %	61.3	89.1
Food energy, kcal	137.	38.
Protein, g	6.2	1.5
Carbohydrate, g	30.8	8.7
Fat, g	0.2	0.1
Ca, mg	29.	27.
P, mg	202.	36.
Fe, mg	1.5	0.5
Na, mg	19	10.
K, mg	529	157.
Vitamin A, IU	Trace	40.[1]
Thiamin, mg	0.25	0.03
Riboflavin, mg	0.08	0.04
Niacin, mg	0.5	0.2
Ascorbic acid, mg	15	10

[1]Value based on yellow-fleshed varieties, white-fleshed varieties contain only a trace.

dose administered averaged 0.2 ml of garlic oil per patient. The study consisted of three groups: Group 1 included healthy subjects with no history of MI, Group 2 consisted of patients with a history of MI at least one year old, Group 3 included patients with acute MI. After three months, garlic increased ($P \leq 0.001$) FA by 130% in Group 1 and by 83% in Group 2. In Group 3, FA increased ($P \leq 0.001$) to 63% and 95.5% above post-infarct levels after 10 and 20 days. Acute MI patients receiving no garlic experienced a spontaneous increase ($P \leq 0.05$) in FA of about 24%. Upon discontinuation of garlic, FA dropped off sharply and reached the same level as the control patients.

Recognized risk factors associated with CHD appear to be related to FA. FA measurements from a total of 915 men and women in an industrial

population revealed that FA decreased significantly in men as age increased; FA was less in smokers than non-smokers; and mean FA for 10 men with a history of MI was significantly less than in men free of ischemic heart disease. FA was also observed to be negatively correlated with blood pressure and cholesterol and triglyceride levels in the blood (46).

Platelet aggregation. Both garlic (20) and onion (47, 48) have been reported to inhibit platelet aggregation in vitro. Bordia (48) observed that garlic inhibited the aggregation of platelets induced by ADP, epinephrine, and collagen in 6 healthy adults when garlic oil was added directly to the blood and also in blood drawn from patients 5 days after administering 25 mg garlic oil/day. Makheja et al. (49, 50) indicated that onion and garlic inhibit platelet aggregation by blocking the conversion of arachidonic acid to thromboxane through the inhibition of cyclo-oxygenase. This is the same mechanism by which aspirin and indomethicin affect platelet aggregation (41).

Platelet aggregation may play a significant role in the thrombotic process. Patients with Type II hyperlipidemia showed an increased platelet aggregation with ADP, epinephrine and collagen (51). Renaud observed that rats fed saturated fats experienced an increased aggregation to thrombin (52) and platelet aggregation in epidemiological studies has been positively and significantly correlated to saturated fat intake (53).

Alimentary lipemia

The protective action of whole, boiled and fried onions on changes normally occurring during alimentary lipemia has been reported by Gupta et al. (54), Menon (55, 56) and Mittal et al. (57). Only Bordia et al. (4) verified similar effects by garlic.

Bordia et al. (44) reported a significant increase in plasma cholesterol and a significant decrease in fibrinolytic activity and whole blood coagulation time in 10 healthy adult males aged 30-50 years within 3 hours of ingestion of a meal containing 100 g of butter. When freshly extracted garlic juice (obtained from 50 g garlic) was added to the fatty meal a decrease ($P \leq 0.001$) in both plasma cholesterol and plasma fibrinogin along with an increase ($P \leq 0.001$) in whole blood coagulation time and fibrinolytic activity ($P \leq 0.01$) were observed. Ether extracted garlic oil (from 50 g garlic) produced similar results when administered to the subjects which indicated that the active principle is contained within the oily fraction. The addition of onion juice and onion oil produced similar but slightly lesser effects as compared to the garlic.

The mechanism by which garlic and onion affects butter-induced alimentary lipemia in man is unknown. Sharma et al. (58) reported that 50 g of raw onion reduced ($P \leq 0.001$) serum cholesterol even when it was administered 2 hours after the fatty meal. According to Sharma et al., these results suggested that onion does not interfere with fat absorption, but acts on some other metabolic pathway of fat utilization in the body.

Hypolipemic effects of garlic in rabbits

Experimentally induced atherosclerosis in the cholesterol fed rabbit has produced an important model for the investigation of the effects of garlic on factors associated with CHD. Cholesterol feeding in rabbits has produced hypercholesterolemia, increased serum triglycerides and phospholipids, increased tissue cholesterol, atheromatous changes in the aorta and decreases in fibrinolytic activity (17, 42).

Serum lipids. Many of the initial studies of garlic's hypolipemic action have utilized garlic juice. Jain (59) reported that daily gastric intubation of 10 ml of garlic juice (expressed from 25 g of garlic) lowered ($P \leq 0.001$) serum cholesterol values by 80%, from 2150.7 mg/dl in cholesterol fed rabbits to 418.7 mg/dl in animals fed cholesterol and garlic juice. Garlic feeding, however, did not return cholesterol levels to the 75.5 mg/dl mean of control animals receiving no cholesterol.

Jain (59) reported no significant reduction of serum cholesterol using 10 ml of onion juice (expressed from 25 g of onion). However, Sharma et al. (58) observed that daily gastric intubation of onion juice (equivalent to 25 g/kg) reduced ($P \leq 0.01$) serum cholesterol values whether it was administered at the same time cholesterol feeding was initiated or whether a hypercholesterolemic state had been attained previously in the rabbit by 8 weeks of cholesterol feeding.

Sainani et al. (42) also studied the effects of gastric intubation of garlic juice (0.25 g/day) and onion juice (2.5 g/day) dispersed in 10 ml of distilled water on hypercholesterolemic rabbits for a period of 16 weeks. Both garlic and onion restricted ($P \leq 0.001$) the increase in serum cholesterol ($P \leq 0.001$), triglycerides ($P \leq 0.001$) and phospholipids ($P \leq 0.05$) compared to the animals receiving only cholesterol. Quantitatively, garlic was more effective than onion despite the greater amount of onion juice administered.

Once the hypocholesterolemic actions of garlic juice were reported, further investigations were conducted using a more pharmacologically refined product of garlic, i.e. garlic oil. Jain (60) prepared an alcoholic extract of garlic using diethyl ether (95%) and administered garlic oil (obtained from 0.25, 0.5 and 1.0 g garlic) daily to cholesterol fed rabbits for 16 weeks.

The lowest dose did not significantly alter serum cholesterol levels, but the 0.5 g dose reduced ($P \leq 0.05$) serum cholesterol by 22% and 1.0 g of garlic oil produced a 30% reduction ($P \leq 0.001$) of serum cholesterol. Additional studies by Jain (22, 61) employing the same doses of garlic have produced comparable results.

Bordia et al. (17) observed that essential oils of garlic (extracted from 1 g raw garlic/kg) and onion (extracted from 2 g raw onion/kg) reduced ($P \leq 0.001$) serum cholesterol after 2 and 4 months of cholesterol feeding (0.5 g/kg) in rabbits. Garlic, again, was more effective ($P \leq 0.001$) than onion in lowering serum lipids, reducing cholesterol by 28% compared to 12% for onion and lowering serum triglycerides by 48% compared to 20% for onion.

Free and ester cholesterol. Cholesterol feeding in rabbits has been shown not only to increase total serum cholesterol but also to alter the normal ratio of free cholesterol (FC) to ester cholesterol (EC). In a 16 week study, Jain and Konar (22) observed that the addition of 2 g cholesterol/day to the diets of male albino rabbits produced an increase of 33% in total cholesterol, 23% in FC and 36% in EC. The comparatively greater increase in the EC fraction than total or FC can be expressed as a decrease in the FC/EC ratio. The FC/EC ratio decreased from 0.58[1] at the beginning of the experiment to 0.335 after 16 weeks.

The administration of garlic oil (obtained from 1 g of garlic) did not totally prevent hypercholesterolemia; a 22% increase in total serum cholesterol was observed in garlic supplemented animals compared to a 33% increase in animals fed cholesterol but no garlic. However, the FC/EC ratio

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[1]The results section of Jain's paper gives initial FC/EC as 0.48, but data in their tables list FC/EC as 22.6/40.1 mg cholesterol/dl, therefore a ratio of 0.58.

actually increased in cholesterol fed, garlic supplemented animals from an initial ratio of 0.53 to a final ratio of 0.66, indicating the rise in the EC fraction was more inhibited than that of the FC fraction. Jain and Konar (22) suggested that the protective action of garlic may lie in this inhibiting action of the EC fraction since it is the EC form that accumulates in the intima of cholesterol fed rabbits (62).

Serum lipoproteins. Bordia et al. (17) observed that cholesterol feeding in rabbits produced a rise in the β/α lipoprotein ratio. An initial ratio of 1.3:1 increased to 4.5:1 in 2 months and rose to 5.7:1 after 4 months. The β-lipoproteins and pre-β-lipoproteins were increased by 29.7% and 185%, respectively, while α-lipoproteins decreased by 69.4%.

The addition of garlic and onion oil to cholesterol fed rabbits stabilized the β/α ratio at 1.7:1 and 2.1:1, respectively. The increase in β-lipoproteins was restricted ($P \leq 0.01$) to 5.4% in garlic supplemented animals and 7.2% in onion fed rabbits, but the cholesterol induced increase in pre-β-lipoproteins was not significantly affected by onion or garlic. The normalization of the β/α lipoprotein ratio by garlic was primarily due to its effect on the α-lipoproteins. Garlic and onion restricted ($P \leq 0.001$) the fall in α-lipoproteins to only 21.2% in garlic and 35% in onion fed animals compared to animals receiving cholesterol only.

Sainani et al. (42) also determined the effect of garlic on serum lipoproteins of hypercholesterolemic rabbits and reported results comparable to those of Bordia et al. (17). Cholesterol feeding effected a 31% increase in β-lipoproteins and a 73% decrease in α-lipoproteins, producing a β/α lipoprotein ratio of 11.5:1. Garlic supplementation to cholesterol fed rabbits restricted the rise in β-lipoproteins to only 5.7% and restricted

the decrease of α-lipoproteins to only 14%, producing a β/α lipoprotein ratio of 0.28 comparable to the 2.3 ratio of control animals receiving no cholesterol.

Atherosclerotic lesions and liver lipids. A hypolipemic action alone is not sufficient evidence that an agent is producing beneficial changes in experimentally induced atherosclerosis since a decrease in serum lipids may be created by an increased deposition of those lipids in aortic lesions or tissue stores (14). The majority of studies involving the hypocholesterolemic effects of garlic also have been concerned with the action of garlic on atherosclerotic lesions induced by cholesterol feeding in the rabbit for 16 weeks (17, 21, 22, 42, 58, 60). The results of all the studies indicated that garlic was effective in reducing the degree of atherosclerosis in cholesterol fed rabbits.

Jain and Konar (22) reported that garlic oil (equivalent to 1 g garlic) reduced ($P \leq 0.001$) the cholesterol content of the aorta by 40%, from 32.9 mg/g in cholesterol fed animals to 19.7 mg/g in cholesterol fed rabbits supplemented with garlic oil. Aortic atherosclerosis also was graded grossly on a scale of 0 to 4. Grade 0 indicated that atherosclerotic involvement did not exceed 2% of the total surface of the aortic intima. Grade 1 represented 2-10% involvement; Grade 2, 11-20%; Grade 3, 21-40%; and Grade 4, 41-80% (42). Cholesterol fed rabbits received a mean atheroma score of 2.9 which was reduced ($P \leq 0.001$) to 1.4 in animals receiving garlic. Bordia (17) and Kritchevsky (63) observed similar 50% reductions in atherosclerotic grading of rabbit aortas with the addition of garlic oil. Jain and Konar (22) reported that a dose of 0.5 g garlic oil also reduced ($P \leq 0.02$) the atheroma score to 2.10 but 0.25 g garlic oil produced no significant difference in atherosclerotic lesions compared to rabbits receiving only cholesterol.

Jain and Konar (22) also reported that 1.0 g garlic oil reduced ($P \leq 0.01$) the accumulation of cholesterol in the liver by 23.7%, from 93.7 mg/g in cholesterol fed rabbits receiving no garlic to 73.5 mg/g in cholesterol fed rabbits supplemented with garlic. These results were confirmed in an additional study by Jain (60) when a 26.9% reduction ($P \leq 0.001$) in liver cholesterol was produced by the administration of garlic oil to rabbits.

Comparison with clofibrate. One method of judging the effectiveness of a lipid-lowering agent is to compare its effectiveness with a standard drug. Bordia et al. (27) compared the effects of garlic and onion oil (obtained from 1 g raw onion and garlic) on serum and tissue lipids of cholesterol fed rabbits with that of clofibrate (33 mg/kg), a drug with known hypolipemic action (64). After a 12 week experimental period, garlic, onion and clofibrate reduced serum cholesterol values by 19.8%, 14.6% and 7.9%, respectively. The mean normal lipid content of the aorta, 5.95 mg/100 mg dry weight, was doubled to 13.75 mg by cholesterol feeding. The aortic lipid content of cholesterol fed rabbits supplemented with all three agents was lowered ($P \leq 0.001$), producing aortic lipid levels of 5.28 mg in garlic, 6.23 mg in onion and 7.79 mg in clofibrate groups. Garlic was not only more effective in reducing ($P \leq 0.001$) the aortic lipid accumulation compared to clofibrate, but the aortic lipid level of garlic fed animals was slightly less than that of the control group receiving no cholesterol.

In a second 16 week study comparing the effects of garlic and onion oil to clofibrate, Bordia et al. (21) observed that garlic and onion prevented ($P < 0.01$) a rise in β-lipoproteins and prevented ($P < 0.001$) a fall in α-lipoproteins. The order of effectiveness of each agent in lowering serum cholesterol, serum triglycerides and lipid content of the aorta was: garlic, onion, and clofibrate.

Regression of atherosclerotic lesions. One of the most exciting prospects of atherosclerotic drug research is the discovery of agents with the potential to reverse advanced atherosclerotic lesions. Bordia and Verma (65) investigated the effect of garlic on the reversibility of experimental atherosclerosis induced by feeding cholesterol to rabbits for 16 weeks. When cholesterol was discontinued, one half of the animals received 2 g garlic oil/kg/day and the remainder served as controls. Serum cholesterol rose ($P \leq 0.05$) in the first 2 months of garlic feeding and then dropped sharply. They maintained that the initial rise may be attributed to mobilization of cholesterol from arterial walls or deposits from the liver. Feeding garlic also resulted in decreased serum triglycerides, reduced β/α lipoprotein ratio from 5.2:1 to 2.5:1 and increased ($P \leq 0.001$) fibrinolytic activity of the blood. Aortic examination revealed a mean score of 0.5 for garlic fed animals and 2.5 for controls.

Hypolipemic effects of garlic in rats

Augusti and Matthew (66) conducted a study of the hypocholesterolemic effects of garlic using rats with normal lipid levels maintained on laboratory feed as an experimental model. Freshly prepared aqueous extracts of onion and garlic (1 ml/100 g body weight; 1:3, garlic juice to water) were administered to young male Wistar rats averaging 130 g for a 2 month period. Although no statistical analyses were presented, garlic feeding produced reductions of 15.4% in serum cholesterol (from 52 mg/dl to 44 mg/dl), 11.7% in liver cholesterol, 29% in total serum lipids, and 23% in serum phospholipids. However, unlike results obtained from hypercholesterolemic rabbits (22), garlic was more effective in decreasing the FC fraction than the EC fraction. The control group had a FC/EC ratio of 0.70 and garlic fed

animals had a ratio of 0.57. The authors contended that the relatively greater decrease in FC compared to EC indicates an increase in the esterification of FC leading to better transportation and utilization of lipids. However, the fact that the rats were not hypercholesterolemic may account for the difference in results between this study and the studies involving hypercholesterolemic rabbits.

Itokawa et al. (30) investigated the effects of two sulfur-containing amino acids extracted from garlic, S-methyl-L-cysteine (SMCS) and S-allyl-L-cysteine (SACS) on serum lipids of hypercholesterolemic young male Wistar rats. SMCS and SACS were added at the 0.5% level of the diet. Rats supplemented with SMCS and SACS showed lower ($P \leq 0.01$) serum and liver cholesterol concentrations than those of control animals. Serum phospholipids and cholesterol levels of the aorta revealed no significant differences among the groups.

Hypolipemic action of garlic in human beings

Results of two human studies on the hypolipemic action of garlic indicated that the effectiveness of garlic, as seen in animal studies (22), is related to the dose administered. For a period of 3 weeks, Jain (43) included 5.0 g of crushed garlic bulbs in the diets of 6 male subjects aged 25-30 years with initial mean serum cholesterol values of 227.6 mg/dl. The addition of garlic lowered serum triglycerides and increased FA of the blood at the end of the second ($P \leq 0.05$) and third ($P \leq 0.01$) weeks. Serum cholesterol and β-lipoprotein cholesterol were not significantly affected by garlic, although a lowering trend was noted.

Augusti (67) fed a greater amount (10 g) of garlic than Jain (43) had fed (5 g) to human subjects. In a 2 month study, daily oral administration

of an aqueous extract of garlic (0.5 ml/kg) to 4 male and 1 female hypercholesterolemic patients reduced ($P \leq 0.01$) serum cholesterol levels by 28.5%, from a mean of 305 mg/dl to 218 mg/dl. The subjects consumed an average of 30 ml garlic extract that had been obtained from 10 g of garlic. In 2 patients who discontinued treatment after the end of the experimental period, serum cholesterol returned to initial values after one month.

Epidemiological studies

Sainani et al. (68) conducted an epidemiological study of 3 community groups who varied in their consumption of onion and garlic in the Jain community in Poona. Group 1 included 45 people on a mixed, but primarily vegetarian diet, who regularly consumed 600 g or more onion and 50 g or more garlic/week. Group 2 consisted of 35 strict vegetarians who did not eat garlic or onion. Group 3 included 33 people who were blood relatives of Group 2 and who were also strict vegetarians but who consumed smaller amounts of onion, 200 g/week or less and garlic, 10 g/week or less than Group 1. There were no significant differences in serum cholesterol values or blood coagulation times among the groups. Group 1, who consumed the most garlic and onions, had lower serum triglycerides ($P \leq 0.01$) and β-lipoproteins ($P \leq 0.05$) than Groups 2 or 3. Group 2, who consumed no onion or garlic, had higher phospholipid ($P \leq 0.05$) and fibrinogin ($P \leq 0.01$) values than Groups 1 or 3. Sainani et al. concluded that regular consumption of onion and garlic in the diet had a protective effect on factors associated with atherosclerosis. Baghurst et al. (48) questioned the strength of this statement due to a number of other variables within these groups that may also affect serum lipid values, such as adiposity, exercise, fat intake and conditions of taking the blood samples.

Dietary surveys associated with international epidemiological studies have not included a measurement of garlic consumption. However, Buck et al. (69) studied the relationship of garlic and onion consumption (Table 3) to ischemic heart disease mortality rates available in the U.N. Demographic Year Book. Multiple regression analysis of ischemic heart disease mortality rates among men aged 55-64 years in 1973 on the combined consumption of onion and garlic showed no significant correlation. A significant negative correlation was found for wine consumption and a significant positive correlation was found for intake of animal fat.

Mechanism of action

The mechanism of action by which garlic lowers serum cholesterol is unknown, but several possibilities have been suggested. Investigators generally agree that the action of garlic is associated with the sulfur containing compounds naturally present in garlic.

Augusti (67) suggested that the hypocholesterolemic action of garlic may be related to a decrease in cholesterol biosynthesis. The unsaturated allyl side chains of diallyl thiosulfinate might be reduced easily to saturated propyl side chains and thereby lower the amount of NADH and NADPH in the body available for cholesterol biosynthesis.

Investigations by Small et al. (70) on the antibacterial properties of garlic showed a marked reactivity of the sulfoxide ($\overset{S-S}{\underset{O}{\|}}$) group of diallyl thiosulfinate and synthetic alkyl thiosulfinates to the sulfhydryl (-SH) groups of cysteine. According to Augusti (67) diallyl thiosulfinate might block the sulfhydryl group of CoA, an essential compound in the biosynthesis of cholesterol. A decrease in availability of essential compounds, such as ATP, NADPH and acetyl CoA, has been shown to decrease cholesterol biosynthesis in man during starvation (71).

Sulfur-containing amino acids, i.e. methionine and cysteine, also have been reported to decrease serum cholesterol levels (72) but their effect is not as great as the compounds found in garlic (30). The lipid lowering effect of those essential sulfur-containing amino acids has been attributed to an increase in fecal sterol and cholic acid excretion (30). Itokawa et al. (30) cited a study utilizing ^{14}C-labeled cholesterol in mice which indicated that the hypocholesterolemic action of sulfur-containing amino acids found in garlic was also due to an increase in fecal sterol and cholic acid.

MATERIALS AND METHODS

Animal care

Forty Sprague-Dawley male weanling rats[1] weighing 50-66 g were obtained fro the 7 week study. Animals were assigned randomly to individual, suspended cages with screen bottoms in a room maintained at 21-24°C with a 12 hour light cycle. Feed and de-ionized water were provided ad libitum. Feed intake and weight gain were recorded weekly for individual animals. Feed waste was collected daily and accounted for in feed intake calculations.

Experimental design and diets

The animals were divided randomly into 4 groups of 10 rats in a 2 x 2 factorial design. The four groups consisted of:

Group 1 Low fat beef tallow diet; no garlic

Group 2 Low fat beef tallow diet; garlic

Group 3 High fat beef tallow diet, no garlic

Group 4 High fat beef tallow diet, garlic

The composition of the diets are given in Table 5. In the low fat diet, fat contributed 12.1% of the calories. In the high fat diet, 39.5% of the calories were derived from fat. Modifications in fat were made by varying the amount of dextrose. Ingredients for each diet were mixed in a Hobart automatic mixer for 30 minutes. Mixed diets were stored in gallon-sized plastic containers or individual quart-sized glass jars in the refrigerator.

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[1]Purchased from ARS/Sprague-Dawley, Gibco Animal Resource Laboratories, Madison, Wisconsin 53711

TABLE 5

Composition of basal diet

Ingredients	Low fat Diet	High fat Diet
	(g/100g)	(g/100g)
Casein	20.0	20.0
DL-methionine	0.2	0.2
Corn Oil	2.0	2.0
Beef Tallow	2.0	17.0
Dextrose	63.6	48.6
Vitamin mix[1]	2.2	2.2
Mineral mix[2]	5.0	5.0
Cholesterol	1.0	1.0
Alphacel	5.0	5.0

[1]Vitamin diet fortification mixture #904654 (supplied by Nutritional Biochemicals Division, ICN Pharmaceuticals, Inc.) (in mg/kg diet): vitamin A (200,000 IU/g), 14.85; vitamin D_3 (400,000 IU/g), 0.825; alpha-tocopherol, 16.5; ascorbic acid, 148.5; inositol, 16.5; choline chloride, 247.5; manadione, 7.425; p-aminobenzoic acid, 16.5, niacin, 14.85; riboflavin, 3.3; pyridoxine hydrochloride, 3.3; thiamin hydrochloride, 3.3; calcium pantothenate, 9.9; biotin, 0.066; folic acid, 0.297; and, vitamin B_{12}, 0.0045.

[2]AIN mineral mixture 76™ (supplied by Nutritional Biochemicals Division, ICN Pharmaceuticals, Inc.) (in g/kg mixture): calcium phosphate, dibasic (CaHPO), 500; sodium chloride (NaCl), 74; potassium citrate, monohydrate $(HOC(C)^4)K)(CH_4COOK)_4 \cdot H_2O$, 220; potassium sulfate (K_2SO_4), 52; magnesium oxide (MgO), 24; manganous carbonate (43-48% Mn), 3.5; ferric citrate (16-17% Fe), 6; zinc carbonate (70% ZnO), 1.6; cupric carbonate (53-55% Cu), 0.3; potassium iodate (KIO_3), 0.01; sodium selenite ($Na_2SeO_3 \cdot 5H_2O$), 0.01; chromium potassium sulfate ($CrK(SO_4)_2 \cdot 12\ H_2O$, 0.55; sucrose, finely powdered, 118.

Garlic was administered to the animals in the form of FCC (Food Chemicals Codex) grade garlic oil[1] (28). Garlic-fed animals, Groups 2 and 4, received 0.01 µl of garlic per gram body weight daily suspended in 0.2 ml of corn oil. Weekly mean body weights determined the actual dosage given. The garlic-corn oil mixture was administered daily by gastric intubation using 20 gauge, 1 1/2 inch stainless steel feeding needles.[2] Groups 1 and 3 did not receive garlic oil, but were administered 0.2 ml of corn oil daily by gastric intubation.

Autopsy procedure

After fasting overnight, the animals were sacrificed by intraperitoneal injection of a chloral-hydrate-magnesium sulphate anesthesia, A. L. Thesia[3] (0.3cc/100 g). Blood was drawn via the abdominal aorta and stored in the refrigerator for 15 minutes to facilitate clot retraction. Serum was obtained by centrifugation at 1500 rpm for 15 minutes. The liver and heart were immediately excised, rinsed in normal saline solution, blotted and weighed. The tissues were then wrapped in foil, frozen in liquid nitrogen and stored at -20°C until analyzed. Serum was refrigerated until HDL cholesterol was determined, then frozen for further analyses.

Analytical methods

All analyses were carried out in the Foods and Nutrition laboratories, Justin Hall, Kansas State University. Full descriptions of the methods are given in the Appendix.

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[1]Purchased from Fritzche, Dodge & Olcott, Inc., N.Y., N.Y. 10011

[2]Obtained from Popper & Sons, Inc., New Hyde Park, N.Y. 11040

[3]Obtained from Haver-Lockhart Laboratories, Kansas

Total serum cholesterol was determined by the method of Parekh and Jung as published in Clinical Laboratory Methods (73). The free cholesterol method of Schoenheimer and Sperry as published in Hawk's Physiological Chemistry (74) was used. Esterified cholesterol was determined by the following calculation: Total cholesterol - free cholesterol = esterified cholesterol. Serum Triglycerides were determined by the method of Fletcher as published in Clinical Laboratory Methods (73). HDLs were separated from the serum by a modification of the method of Lopez-Virella et al. (75) and liver lipids were extracted using chloroform, methanol and sodium sulfate. The HDL extract and liver lipid extract were subjected to cholesterol determination as described under total serum cholesterol analysis.

Statistical Analysis

Data from all measurements were subjected to analysis of variance, and the means were separated by Fischer's LSD with P=0.10, 0.05, and 0.01 as appropriate when the F-test rejected the hypothesis of equal means. Weight gain was adjusted linearly for feed intake in covariant analysis, following by t-tests on adjusted means.

RESULTS AND DISCUSSION

Feed intake and weight gain

The effects of fat level in the diet and administration of garlic oil on unadjusted feed intakes and weight gains and weight gains adjusted for feed intake are presented in Table 6. Analyses of variance of these factors are presented in Table 7 and analysis of covariance in Table 8.

Rats fed the low fat diet (4% by weight) had higher ($P \leq 0.01$) feed intakes but gained less ($P \leq 0.01$) weight than rats fed the high fat diet (19% by weight). Both diets contained 1% cholesterol. Similar findings were reported by Chang and Johnson (23). Since there was a high correlation ($r=0.607$, $P \leq 0.01$) between weight gain and feed intake of the rats, weight gain was adjusted linearly for feed intake in covariant analysis. The adjusted weight gain of the high fat group was also more ($P \leq 0.01$) than that of the low fat group.

Supplementation with garlic did not significantly affect unadjusted feed intake or weight gain. However, when weight gain was adjusted for feed intake, the weight gain of the garlic-supplemented rats was lower ($P \leq 0.10$) than that of the non-supplemented animals. Itokawa et al. (30) and Augusti and Mathew (70) observed similar feed consumption and unadjusted weight gains in rats administered garlic extracts and control animals receiving no garlic. Analysis of covariance with feed intake as the covariant was not presented in either study. No undesirable effects from garlic or garlic extract administration to animals or human beings have been reported in the literature. Bordia et al. (21) found that the administration of garlic oil extracted from 1 g garlic per day to cholesterol fed rabbits did not significantly change

TABLE 6

Effects of fat level in the diet and administration of garlic oil on feed intakes and weight gains[1]

Variation	Unadjusted		Adjusted[2]
	Feed intake	Weight gain	Weight gain
	g	g	g
Fat level			
Low fat diet	806.7	261.7	249.8
	**[3]	**	**
High fat diet	712.9	285.6	297.4
Garlic			
No garlic	759.3	278.1	278.2
	ns	ns	+
Garlic	760.3	269.2	269.0

[1]Numbers represent means of 20 animals. [2]Adjusted by linear regression for feed intake. [3]**, ($P \leq 0.01$), +, ($P \leq 0.10$), ns, not significant.

TABLE 7

Analyses of variance of feed intake and weight gain

Source of Variation	DF	Mean Squares and Significances[1]	
		Feed Intake	Weight gain
Fat level	1	87984.**	5688.**
Garlic	1	10.00^{ns}	801.0^{ns}
F X G	1	$1988.^{ns}$	126.0^{ns}
Rats	36	1688.	324.2

[1]**, $P \leq 0.01$; ns, not significant.

TABLE 8

Analysis of covariance of weight gain with feed intake as the covariant

Source of Variation	DF	Adjusted mean squares and significance[1] Weight gain
Fat level	1	9179.**
Garlic	1	876.8+
F X G	1	0.002^{ns}
Regression	1	3840.**
Rats	34	223.7
Total	38	

[1] **, ($P \leq 0.01$); +, ($P \leq 0.10$; ns, not significant.

feed intake or body weight and did not produce any immediate effect such as regurgitation or other signs of toxicity.

Organ Weights

The effects of fat level in the diet and administration of garlic oil on liver and heart weight and relative liver and heart size are shown in Table 9, and analyses of variance are presented in Table 10. The animals fed the high fat diet had greater ($P \leq 0.01$) liver and heart weights and relative liver size than the animals fed the low fat diet. No significant difference in relative heart size related to diet was seen. Chang and Johnson (23) also reported higher ($P \leq 0.01$) relative liver weights in animals fed a high fat (20% by weight) compared to a low fat (5% by weight) diet.

The administration of commercial garlic oil in the amounts used in this study did not significantly affect liver and heart weight or relative liver size of the test animals. The relative heart size was lower ($P \leq 0.10$)

TABLE 9

Effects of fat level in the diet and administration of garlic oil on liver and heart weight and relative liver and heart size[1]

Variation	Liver wt.	Heart wt.	Relative Liver size	Relative Heart size[2]
Fat level	g	g	%	%
Low fat	9.64	1.02	3.06	0.325
	**[3]	**	**	ns
High fat	12.29	1.12	3.57	0.326
Garlic				
No garlic	11.21	1.07	3.33	0.333
	ns	ns	ns	+
Garlic	10.72	1.07	3.30	0.318

[1]Numbers represent means of 20 animals. [2]Relative liver and heart size = (organ weight X 100)/(body weight). [3]**, $P \leq 0.01$; +, $P \leq 0.10$; ns, not significant.

TABLE 10

Analyses of variance on liver and heart weight and relative liver and heart size[1]

Source of Variation	DF	Mean Squares and Significances[1]			
		Liver wt.	Heart wt.	Relative Liver size[2]	Relative heart size[2]
Fat level	1	69.96**	0.09216*	2.647**	0.000002^{ns}
Garlic	1	2.430^{ns}	0.00001^{ns}	0.00702^{ns}	0.00221^{+}
F X G	1	0.036^{ns}	0.00441^{ns}	0.01056^{ns}	0.000616^{ns}
Rats	36	2.018	0.009301	0.1044	0.000589

[1]**, $P \leq 0.01$; *, $P \leq 0.05$; +, $P \leq 0.10$; ns, not significant.

in the garlic supplemented group compared to the non-supplemented group. No other researchers have reported the effect of garlic on relative heart size of rats.

Total serum cholesterol and liver cholesterol

Total serum and liver cholesterol means are presented in Table 11 and analyses of variance of these factors are presented in Table 12. Total serum cholesterol values were 23.3% higher ($P \leq 0.01$) and liver cholesterol levels were 37.5% higher ($P \leq 0.01$) in the high fat diet animals compared to lipid levels of the low fat diet group. These results are in agreement with those of other studies (23, 76) which show a relationship between higher dietary fat levels and higher serum cholesterol and liver cholesterol. Human and animal studies have indicated that both the amount and saturation of fat in the diet affects serum and liver lipid concentrations, although the exact mechanism is unknown. Cholesterol absorption is enhanced with a high fat diet and restricted with a low fat diet (77).

Total serum cholesterol was 9.6% lower ($P \leq 0.05$) in animals supplemented with garlic oil than in rats receiving no garlic oil (Table 11). However, the degree by which total serum cholesterol was lower in garlic supplemented rats was moderate compared to other investigations on the hypocholesterolemic action of garlic. Jain (59) reported the daily administration of 10 ml of freshly extracted garlic juice obtained from 25 g garlic resulted in 80% lower total serum cholesterol values in cholesterol fed rabbits compared to lipid values of rabbits fed only cholesterol. Using lower doses, Jain (60) and Bordia et al. (17) observed a 30% and a 28% reduction, respectively, in total serum cholesterol values of cholesterol fed rabbits supplemented daily with garlic oil freshly

TABLE 11

Effects of fat level in the diet and administration of garlic on total cholesterol, HDL cholesterol, HDL/total cholesterol, free, ester and free/ester cholesterol, serum triglycerides and liver cholesterol[1]

Variation	Serum Cholesterol						Serum Triglycerides	Liver Cholesterol
	Total	HDL	HDL/total	Free	Ester	Free/Ester		
	mg/dl	mg/dl		mg/dl	mg/dl		mg/dl	mg/g wet liver
Fat level								
Low fat	83.3	44.0	53.6	13.5	69.8	19.7	77.3	3.70
	**[2]	ns	*	**	**	ns	**	**
High fat	108.6[3]	47.8[3]	45.3[3]	16.4[4]	91.9[4]	18.0[4]	103.4[3]	5.95
Garlic								
No garlic	100.8	45.7[5]	47.7[5]	15.5[4]	84.5[4]	19.1[4]	94.5[5]	5.07
	*	ns	ns	ns	+	ns	+	ns
Garlic	91.1	45.8[4]	51.8[4]	14.3	76.8	18.6	84.3[4]	4.57

[1]Numbers represent means of 20 animals unless otherwise indicated. [2]**, $P<0.01$; *, $P<0.05$; +, $P<0.10$; ns, not significant. [3]Numbers represent means of 17 animals. [4]Numbers represent means of 19 animals. [5]Numbers represent means of 18 animals.

TABLE 12

Analyses of variance of total cholesterol, HDL cholesterol, HDL/total cholesterol, free, ester and free/ester cholesterol, serum triglycerides and liver cholesterol

Source of Variation	DF	Mean Squares and Significances[1]							
		Serum Cholesterol						Serum Tri-glycerides	Liver Cholesterol
		Total	HDL	HDL/Total	Free	Ester	Free/Ester		
Fat level	1	6429.**	130.4^{ns}	621.1**	84.98**	4763.**	27.51^{ns}	6276.**	50.45**
Garlic	1	926.1*	0.0446^{ns}	154.6^{ns}	13.92^{ns}	581.0^{+}	2.24^{ns}	956.8^{+}	2.450^{ns}
F X G	1	241.3^{ns}	0.7934^{ns}	21.78^{ns}	1.880^{ns}	335.3^{ns}	17.81^{ns}	537.8^{ns}	2.798^{+}
Rats	36	184.2	82.65	89.48	7.913	148.5	10.87	297.9	0.9985

[1]**, $P \leq 0.01$; *, $P \leq 0.05$; +, $P \leq 0.10$; ns, not significant.

extracted from 1.0 g garlic. When garlic oil obtained from 0.5 g garlic was administered to cholesterol fed rabbits (60), total serum cholesterol values were 21% lower compared to those of animals fed only cholesterol, and garlic oil from 0.25 g garlic did not significantly affect total serum cholesterol.

Bordia et al. (27) estimated 1.0 g garlic to yield about 0.01 ml garlic oil. In Bordia's studies with cholesterol fed rabbits averaging 1 kg in body weight, the actual dose was 0.01 ml garlic oil/day. The same dosage was used in this study. Therefore, initially the approximately 50 g rats received 0.0005 ml garlic oil/day and at the end of the experimental period the rats averaged 305 g and received 0.00305 ml of garlic oil suspended in 0.2 ml of corn oil/day.

The difference in chemical composition between freshly extracted garlic oil and the commercial garlic oil used in this study may account somewhat for their difference in lipid lowering effects, since similar dosages of each oil were used. Itokawa et al. (30) observed the effect of seven sulfur-containing amino acids at the level of 0.5% of the diet on plasma cholesterol of hypercholesterolemic rats. Two amino acids extracted from fresh garlic, S-methyl-L-cysteine sulfoxide (SMCS) and S-allyl-L-cysteine sulfoxide (SACS) had a more potent effect in reducing ($P \leq 0.01$) total serum cholesterol than the essential amino acid, L-methionine, which produced a lesser ($P \leq 0.05$) decrease in elevated serum cholesterol, or L-cysteine, which did not significantly affect serum cholesterol. Itokawa et al. (30) suggested the sulfoxide groups of SMCS and SACS may affect the methyl- or allyl-cysteine portion of these amino acids and decrease serum cholesterol by some unknown mechanism. Augusti (67) also considered the role of the sulfoxide groups present in garlic extracts important in the

hypocholesterolemic action of garlic. According to Brodnitz et al. (33), the sulfoxide compounds are no longer present in commercial garlic oil, and this lack of sulfoxide groups may account for the less significant serum cholesterol reduction observed in this study compared to studies utilizing freshly extracted garlic juice or oil. Increased doses of commercial garlic oil might produce more significant changes in total serum cholesterol.

Augusti (67) reported that local physicians in Trivandrum, India are advising hypercholesterolemic patients to use 10-15 g garlic per day to reduce serum cholesterol levels. Augusti observed that daily ingestion of about 30 ml of a freshly prepared aqueous extract of garlic (obtained from 10 g raw garlic) reduced serum cholesterol levels by 28.5% in five hypercholesterolemic patients. However, the ingestion of 30 ml of garlic extract per day may not be acceptable to all hypercholesterolemic patients. The advantage of freshly extracted garlic oil and commercial garlic oil over fresh garlic in lowering serum lipids is that a relatively large amount of garlic can be reduced to a small quantity of oil and the somewhat disagreeable odor may be masked by incorporation into a gelatin capsule (21).

No significant difference in liver cholesterol related to garlic administration was observed. However, an interaction between fat level of the diet and garlic supplementation was noted (Table 12). Garlic was effective in lowering ($P \leq 0.10$) liver cholesterol in animals fed the low fat diet, but did not affect liver cholesterol in the high fat diet animals (Table 13).

Chang and Johnson (23) noted that the effectiveness of pectin in lowering serum and liver cholesterol was also higher when rats were fed a low fat diet compared to a high fat diet. Human studies on the

TABLE 13

Effects of administration of garlic oil on liver cholesterol

Variation	Liver cholesterol	
	low fat diet	high fat diet
	mg/g wet liver	mg/g wet liver
No Garlic	4.21[1]	5.93
	+[2]	ns
Garlic	3.19	5.96

[1]Numbers represent means of 20 animals. [2]+, $P<0.10$, ns, not significant.

hypocholesterolemic effects of garlic have been conducted by researchers in India (43, 67), where people consume a lower fat diet than is observed in the United States. The actual dose of garlic administered to hypercholesterolemic patients may need to be varied according to the level of fat in their diet.

HDL cholesterol, HDL/total cholesterol and free, ester and free/ester cholesterol

HDL (high-density or α-lipoprotein) cholesterol, HDL/total and free, ester and free/ester cholesterol means are shown in Table 11. Analyses of variance of these factors are presented in Table 12.

No significant difference in HDL cholesterol of serum was attributed to level of fat in the diet, but the high fat diet produced a decrease ($P<0.05$) in the HDL/total cholesterol ratio compared to the low fat diet. Other investigators have noted that an increase in serum cholesterol in rats (16) and rabbits (17) is associated with a decrease in HDL and an increase in VLDL (very-low-density or pre-βlipoprotein) and LDL (low-density of β-lipoprotein) in serum.

The addition of garlic oil in the amount used in this study did not significantly affect HDL cholesterol or HDL/total cholesterol ratio. Bordia et al. (17) and Sainani et al. (42) reported the addition of garlic oil to cholesterol fed rabbits restricted the decrease in HDL induced by cholesterol feeding and normalized the lipoprotein ratio.

Epidemiological studies have established an inverse relationship between HDL and risk of CHD (7), although a causal relationship has not been determined. Carlson and Ericsson (78) noted the mean values for HDL cholesterol were reduced ($P \leq 0.001$) in myocardial infarction (MI) patients compared to those of control patients. Subnormal values for HDL cholesterol were observed in 39% of the MI patients. A progressive decrease in serum HDL cholesterol values were reported in a longitudinal study of 400 West Point graduates (79). HDL cholesterol values can be increased with the administration of estrogen (80) and HDL concentrations are 30-60% higher in pre-menopausal women compared to those of male counterparts (81). HDL is proportional to exercise and inversely related to obesity, cigarette smoking and VLDL (82).

Gotto et al. (83) described two methods by which HDL might protect against atherosclerosis; first, by competition with LDL for uptake by cells in the arterial wall and second, by serving as a transport vehicle for the removal of cholesterol from peripheral tissues back to the liver.

Researchers are continuing to discover new agents able to modify serum lipoprotein composition. Previous studies with cholesterol fed rabbits (17, 42) indicated that garlic can affect serum lipoproteins by minimizing the increase in HDL induced by cholesterol feeding. No significant change in the HDL fraction was observed in this study related to the administration of

garlic, but the relatively small 9.6% reduction in total serum cholesterol may not have allowed a significant change in the HDL value to be evident. No human studies on the effect of garlic oil on HDL cholesterol have been reported, but further investigation is warranted.

Serum levels of both free and ester cholesterol were increased ($P \leq 0.01$) in animals fed the high fat diet as compared to the low fat diet. The free/ester cholesterol ratio was not significantly affected by the dietary fat level, which indicated that the levels of free and ester cholesterol were similar.

The addition of garlic in this study did not significantly affect free cholesterol levels in the serum but ester cholesterol was lower ($P \leq 0.10$) compared to that of rats receiving no garlic. This decrease in ester cholesterol, however, was not sufficient to significantly affect the free/ester cholesterol ratio.

Augusti and Mathew (66), however, reported that the addition of an aqueous extract of garlic to rats with normal lipid values produced 26% lower free cholesterol values and only 8% lower ester cholesterol values as compared to those of control rats. Results from studies with hypercholesterolemic rabbits administered garlic agree with the findings of the present study; i.e., garlic was more effective in decreasing ester cholesterol than free cholesterol (22).

Serum triglycerides

Tables 11 and 12 show that serum triglyceride concentrations of animals fed the high fat diet were higher ($P \leq 0.01$) than those of rats fed the low fat diet. The ability of high levels of dietary fat to produce

significant increases in serum lipids, including serum triglycerides, is well established.

Serum triglyceride levels are elevated in most of the human hyperlipoproteinemia classifications: Type I, IIb, III, IV and V. The relationship between serum triglycerides and CHD, however, is complex. Epidemiological studies such as the Framingham study did not identify serum triglycerides as an independent risk factor of CHD (7).

Serum triglycerides of garlic-supplemented rats in this study were lower ($P \leq 0.10$) than those of the non-supplemented animals. Other researchers have reported similar results. Sainani et al. (68) and Bordia et al. (17) observed serum triglyceride concentrations in garlic-supplemented rats were lower ($P \leq 0.001$) than those of rabbits receiving no garlic.

SUMMARY

The effect of the administration of garlic oil on serum and tissue lipids of forty male Sprague-Dawley rats fed diets with two levels of beef tallow were investigated during a 7-week feeding study. The 4 experimental groups were: low fat (4% by weight) diet, no garlic; low fat diet, garlic; high fat (19% by weight) no garlic; high fat diet, garlic. The diets were supplemented with 1% cholesterol. Garlic-fed animals received 0.01 µl of commercial garlic oil (Food Chemicals Codex grade) per gram of body weight per day. The garlic oil was suspended in 0.2 ml of corn oil and administered by gastric intubation. Animals not receiving garlic oil were administered 0.2 ml of corn oil daily by gastric intubation. The following measurements were made: feed intake, weight gain, liver and heart weight, total serum cholesterol, HDL cholesterol, free cholesterol, liver cholesterol and serum triglycerides.

Rats fed the low fat diet had higher ($P \leq 0.01$) feed intakes but gained less ($P \leq 0.01$) weight than rats fed the high fat diet. When weight gain was adjusted linearly for feed intake in covariant analysis, the adjusted weight gain of the high fat group also was more ($P \leq 0.01$) than that of the low fat group. Supplementation with garlic did not affect feed intake or unadjusted weight gain. However, adjusted weight gain of the garlic-fed rats was lower ($P \leq 0.10$) than that of the animals not receiving garlic.

The animals fed the high fat diet had greater ($P \leq 0.01$) liver and heart weights and relative liver size than animals fed the low fat diet. No significant difference in relative heart size due to the level of fat in the diet was seen. The administration of garlic did not significantly affect liver and heart weight or relative liver size of the test animals. The

relative heart size was lower ($P \leq 0.10$) for the garlic supplemented group than for the non-supplemented rats.

Total serum cholesterol, liver cholesterol, free and ester cholesterol and serum triglycerides were higher ($P \leq 0.01$) in the high fat diet group than in the low fat diet group. No significant changes in HDL cholesterol or free/ester cholesterol ratio were observed related to fat level in the diet, but the high fat diet did produce a decrease ($P \leq 0.05$) in the HDL/total cholesterol ratio as compared to that of the low fat diet group.

Lower values were seen for total serum cholesterol ($P \leq 0.05$), ester cholesterol ($P \leq 0.10$) and serum triglycerides ($P \leq 0.10$) for animals supplemented with garlic oil than for rats receiving no garlic. Liver cholesterol was not significantly affected by the administration of garlic oil, but analysis of variance indicated an interaction between the level of fat in the diet and garlic. Liver cholesterol for the low fat diet group was lower ($P \leq 0.10$) for garlic supplemented animals than for rats receiving no garlic, but was not affected by garlic in the high fat diet group.

Results from this study show that commercial garlic oil is effective in reducing serum lipids. Further investigations are warranted to define the mechanism by which garlic affects serum lipids and serum lipoproteins.

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APPENDIX

ANALYTICAL PROCEDURES

Total serum cholesterol

Total serum cholesterol was determined by the method of Parekh and Jung as published in Clinical Laboratory Methods (73).

Reagents:

1. Ferric acetate-uranyl acetate solution (FA-UA): Dissolve 0.5 g $FeCl_3 \cdot 6H_2O$ in 10 ml of water in a centrifuge tube. Add 3 ml of concentrated ammonium hydroxide, centrifuge, decant and discard supernatant and wash the precipitate three times with water. Dissolve the precipitate in 1 L of acetic acid, add 0.1 g powdered uranyl acetate and mix well. Allow the solution to stand overnight then mix well again. The solution is stable for 6 months in a brown bottle.
2. Sulfuric acid-ferrous sulfate colution (SA-FS): Dissolve 0.1 g anhydrous ferrous sulfate in 100 ml glacial acetic acid in a 1 L volumetric flask. Add 100 ml of concentrated sulfuric acid while swirling. After cooling to room temperature, dilute to volume with sulfuric acid.
3. Cholesterol standard: Dissolve 250 mg pure cholesterol in chloroform to make 100 ml.

Procedure: A 50 µl portion of serum was added to 10 ml of FA-UA solution to precipitate the proteins and extract the cholesterol. The solution was allowed to stand 5 minutes, then centrifuged at 1700 rpm for 5 minutes. A 3 ml aliquot of the supernatant was transferred to another tube, 2 ml of SA-FS solution was added slowly down the side of the tube, the solution was mixed well and allowed to stand for 20 minutes for color development. The tubes were read against a blank of 3 ml FA-UA and 2 ml SA-FS at 560 nm with a Bausch & Lomb Spectronic 20 spectrophotometer.

Calculation: $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{mg cholesterol/100 ml}$

Serum triglycerides

Serum triglycerides were determined by a modification of the method of Fletcher as published in Clinical Laboratory Methods (73).

Reagents:

1. Zeolite mixture: Grind zeolite in a Waring blender for several minutes then heat at 110°C overnight. Mix 100 g zeolite with 10 g calcium hydroxide, 10 g hydrated aluminum silicate (also called Kaolin or Lloyd's reagent), and 5 g copper sulfate pentahydrate.
2. Potassium hydroxide, 5% 9w/v): Dissolve 5 g potassium hydroxide in isopropanol/water solution (40:60, v/v) to make 100 ml.
3. Sodium metaperiodate, 0.025 M.
   A. Stock solution: Dissolve 5.347 g sodium metaperiodate in 1 L of 1 N acetic acid (58 ml/L water).
   B. Working solution: Prepare fresh daily by diluting 12 ml stock solution and 20 ml isopropanol to 100 ml 1 N acetic acid.
4. Acetylacetone: Dissolve 0.75 ml acetylacetone and 2.5 ml isopropanol in 100 ml 2 M ammonium acetate (154.2 g/L water). The solution is stable for 1 month in a brown bottle in the refrigerator.
5. Triolein standard: Dissolve 120 mg of triolein in isopropanol to make 100 ml. The solution is stable in the refrigerator.

Procedure: A 0.1 ml aliquot of serum or standard was added to 4.9 ml isopropanol. One g of zeolite mixture was added to the serum, standard or blank (5 ml isopropanol) and mixed on a vortex mixer for 20 seconds. The tubes were centrifuged at 2800 rpm for 5 minutes, the supernatant was decanted

into another set of tubes and recentrifuged. A 2 ml aliquot of the supernatant was transferred to capped tubes and 0.6 ml of 5% potassium hydroxide solution was added. The tubes were mixed, capped and incubated for 15 minutes in a 60-70°C water bath. After cooling, 1 ml of working sodium metaperiodate solution was added to each tube, mixed well, and 0.5 ml of acetylacetone solution was added to each tube and mixed well. The tubes were capped and incubated in a 60-70°C water bath for 15 minutes for color development. After cooling, the tubes were read at 405 nm with a Bausch & Lomb Spectronic 20 spectrophotometer.

Calculation: $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{mg triglycerides/100 ml}$

Free Cholesterol

Free cholesterol was determined by the method of Schoenheimer and Sperry as published in Hawk's Physiological Chemistry (74).

Procedure:

A 0.8 ml portion of serum or standard (25 mg cholesterol/100 ml isopropanol) was added to a flask containing 10 ml of acetone-absolute ethanol (1:1). The flasks were covered with aluminum foil and immersed in boiling water until the solvent boiled. After returning to room temperature, 10 additional ml of acetone-ethanol mixture was added. The contents of the flask were mixed well and poured onto dry Whatman filter paper in a covered funnel.

Six ml of the filtrate were transferred to a centrifuge tube and 3 ml of digitonin solution (400 mg/100 ml water) were added along with 3 drops of 10% acetic acid solution. The tube was capped, mixed well and allowed to stand overnight at room temperature. The tube was then centrifuged at

2800 rpm for 15 minutes. The supernatant was removed by gentle suction through a capillary tube without touching the sides of the tube. About 2 ml of acetone-ether (1:1) was added down the sides of the tube, the contents were mixed well, centrifuged for 5 minutes and the supernatant was discarded. The procedure was repeated twice using ether instead of acetone-ether solution. The precipitate was analyzed for cholesterol using the same method as for total serum cholesterol except that 4 ml of FA-UA solution was added to the precipitate, mixed well, and a 3 ml aliquot was used for color determination.

Calculation: $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{Free cholesterol/100 ml}$

High-density lipoprotein cholesterol determination

High density lipoproteins were separated from the serum by a modification of the method of Lopez-Virella et al. (75). All analyses were completed on refrigerated serum within 48 hours of obtaining the serum.

Reagents:

1. Sodium phosphotungstate solution: Dissolve 40 g of phosphotungstic acid per liter of a solution of 1 M NaOH and distilled water (16/84, v/v)
2. $MgCL_2$ (2M)

Procedure:

To 0.5 ml of fresh serum, 0.075 ml of phosphotungstate solution was added, mixed, and 0.0125 ml of $MgCl_2$ was added and mixed well. The tubes were allowed to stand for 5 minutes, then centrifuged at 2500 rpm for 10 minutes and 5000 rpm for 30 minutes. The supernatant was analyzed for cholesterol as described under total serum cholesterol.

Calculation: $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} \times 1.175 = \text{mg cholesterol/}$

100 ml

Liver cholesterol determination

Lipids were extracted from liver by the following procedure: frozen whole livers wrapped in foil were allowed to thaw at room temperature. Two g of tissue were weighed out and placed in a homogenizing tube to which 15 ml of chloroform:methanol (2:1) was added. The liver was homogenized and filtered. An additional 5 ml of chloroform:methanol was added to the homogenizing tube to rinse out any remaining lipids and then filtered.

About 5 ml of distilled water was added to the filtered solvent extract and mixed well. After the phases separated (about 1 minute) the upper phase was removed by gentle suction and discarded. About 5-10 g of sodium sulfate was added to the remaining solvent, mixed and allowed to stand until the phases separated. The upper phase was again removed and discarded. The lower phase was transferred to 25 ml empty flasks and totally evaporated with a gentle flow of nitrogen gas. Three ml of isopropanol were added, mixed well and the solution was placed in teflon capped glass tubes. Prior to cholesterol determination the tubes were gently centrifuged and the upper phase was subjected to cholesterol determination as described under total serum cholesterol determination.

Calculation: $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} = \text{mg liver cholesterol/dl}$

(mg liver cholesterol/dl) X (0.015) - mg cholesterol/g liver

TABLE 14

Initial body, final body weight, total weight gain, feed intake, liver and heart weight, and relative liver and heart size of individual animals

Animal	Body weight			Feed Intake	Organ weight			
	Initial	Final	Total gain		Liver	Relative Liver size	Heart	Relative Heart size
	g	g	g	g	g	g/100g body wt.	g	g/100g body wt.
				Group 1 Low fat diet; no garlic				
1	61	315	254	796	12.295	3.83	1.091	0.348
2	51	305	254	762	8.906	2.99	0.852	0.285
3	58	328	270	823	10.266	3.04	1.124	0.331
4	59	315	256	786	9.918	3.12	0.992	0.311
5	65	352	287	851	10.227	2.96	0.990	0.286
6	59	318	259	784	10.295	3.37	1.108	0.354
7	56	333	277	786	10.548	3.30	0.971	0.303
8	58	305	247	832	8.837	2.82	0.892	0.283
9	58	308	250	744	7.866	2.44	1.075	0.334
10	53	343	290	827	9.840	2.98	1.011	0.306
mean	58	322	264	799	9.920	3.09	1.012	0.314
				Group 2 Low fat diet; garlic				
1	58	302	244	834	9.116	3.20	0.925	0.323
2	59	324	265	900	9.900	3.15	1.295	0.411
3	62	318	256	839	9.223	3.14	0.979	0.333
4	65	339	274	815	9.720	2.83	0.987	0.289
5	57	316	259	787	9.766	3.05	1.103	0.344
6	56	318	262	808	9.108	3.04	0.930	0.310
7	59	356	297	862	11.249	3.21	1.163	0.363
8	52	315	263	749	9.127	2.88	1.084	0.341
9	55	294	239	817	8.349	2.98	0.899	0.321
10	58	289	231	731	8.086	2.78	0.909	0.333
mean	58	317	259	814	9.367	3.03	1.032	0.337

TABLE 14 (continued)

Animal	Body weight			Feed Intake	Organ weight			
	Initial	Final	Total gain		Liver	Relative Liver size	Heart	Relative Heart size
	g	g	g	g	g	g/100g body wt.	g	g/100g body wt.
				Group 3 High fat diet; no garlic				
1	66	350	284	708	13.372	3.70	1.230	0.341
2	53	334	281	690	12.288	3.78	1.073	0.329
3	61	371	310	758	12.651	3.35	1.135	0.299
4	64	349	285	733	13.646	3.79	1.103	0.306
5	50	332	282	682	11.677	3.62	1.110	0.344
6	59	331	272	731	11.211	3.41	1.055	0.322
7	56	359	303	711	11.915	3.51	1.083	0.318
8	58	377	319	767	15.213	4.17	1.212	0.332
9	62	376	314	741	12.219	3.15	1.194	0.306
10	59	327	268	673	10.806	3.19	1.105	0.327
mean	59	350	292	719	12.505	3.57	1.129	0.322
				Group 4 High fat diet; garlic				
1	60	351	291	675	12.011	3.50	1.092	0.318
2	53	314	261	649	9.589	3.15	1.018	0.336
3	50	352	302	749	11.422	3.21	1.257	0.354
4	53	331	278	706	11.372	3.45	1.194	0.361
5	60	352	292	705	15.994	4.48	1.163	0.325
6	64	368	304	750	14.842	3.92	1.190	0.314
7	58	308	250	637	10.435	3.48	0.926	0.310
8	65	356	291	736	13.111	3.64	1.099	0.306
9	63	328	265	770	11.799	3.72	1.093	0.344
10	58	317	259	686	10.149	3.18	1.037	0.326
mean	58	338	279	706	12.072	3.57	1.107	0.329

TABLE 15

Total serum cholesterol, HDL cholesterol, HDL/total cholesterol, free, ester and free/ester cholesterol ratio, serum triglycerides and liver cholesterol of individual animals

Animals	Serum Cholesterol						Serum triglycerides	Liver cholesterol
	Total	HDL	HDL/total	Free	Ester	Free/ester		
	mg/dl	mg/dl		mg/dl	mg/dl		mg/dl	mg/g liver
				Group 1	Low fat diet; no garlic			
1	95.4	38.6	50.5	20.5	74.9	27.4	56.9	3.2
2	60.6	31.6	52.1	11.2	49.3	27.8	92.9	3.3
3	78.8	43.2	54.8	14.1	64.8	21.7	87.3	3.2
4	69.2	38.7	55.9	13.6	55.7	24.4	48.8	4.5
5	94.6	58.3	61.6	13.1	81.6	16.0	75.9	5.9
6	99.8	27.7	27.8	14.3	85.5	16.8	116.3	5.2
7	97.4	60.7	62.3	16.5	80.9	20.4	82.8	4.4
8	89.0	50.4	56.6	13.7	75.3	18.2	63.6	2.6
9	80.5	41.8	52.0	12.9	67.6	19.0	116.6	4.5
10	90.9	48.2	53.0	11.3	79.7	14.1	56.4	5.5
mean	85.6	43.9	51.7	14.1	71.5	20.6	79.7	4.2
				Group 2	Low fat diet; garlic			
11	73.9	40.5	54.8	10.2	63.7	16.0	53.7	3.9
12	78.5	52.9	67.5	13.2	65.2	20.3	97.3	3.2
13	98.7	39.9	40.5	16.7	82.0	20.3	88.6	4.0
14	75.3	43.9	58.3	10.7	64.7	16.5	56.1	4.2
15	80.8	47.9	59.3	11.9	68.9	17.3	85.1	2.6
16	77.4	42.6	57.5	14.6	62.8	23.2	83.1	3.0
17	89.4	27.7	31.0	15.8	73.6	21.4	53.2	4.2
18	76.4	42.5	55.6	12.0	64.4	18.7	57.6	1.9
19	77.9	49.0	62.8	10.7	67.2	15.9	80.6	2.8
20	81.0	54.7	67.5	12.7	68.3	18.5	92.3	2.3
mean	80.9	44.2	55.5	12.8	68.1	18.8	74.8	3.2

TABLE 15 (continued)

Animals	Serum Cholesterol						Serum triglyceride	Liver cholesterol
	Total	HDL	HDL/total	Free	Ester	Free/ester		
	mg/dl	mg/dl		mg/dl	mg/dl		mg/dl	mg/g liver
				Group 3	High fat diet; no garlic			
21	139.7	--	--	22.0	117.8	18.6	120.0	5.7
22	147.7	62.3	42.2	15.4	132.4	11.6	--	6.2
23	99.5	47.8	48.0	14.5	85.0	17.0	89.0	6.1
24	134.3	51.5	38.4	21.4	113.0	18.9	133.8	6.3
25	106.8	47.6	44.6	12.7	94.1	13.5	110.6	6.7
26	109.7	38.0	34.7	20.8	88.8	23.5	110.5	5.6
27	99.6	49.0	49.2	15.5	84.1	18.4	124.8	5.3
28	114.2	--	--	--	--	--	--	4.5
29	100.0	45.2	45.3	14.2	85.7	16.6	111.5	5.3
30	107.5	42.5	39.6	17.5	90.0	19.5	103.2	7.6
mean	115.9	48.0	42.7	17.1	99.0	17.5	112.9	5.9
				Group 4	High fat diet; garlic			
31	99.0	39.2	40.5	13.4	85.6	15.7	86.4	7.0
32	88.1	48.9	55.5	13.0	75.1	17.3	80.0	3.7
33	109.7	47.0	42.9	16.5	93.2	17.7	102.3	7.0
34	86.0	37.8	44.0	12.2	73.8	16.5	103.0	6.0
35	107.3	--	--	18.1	89.2	20.3	--	4.5
36	104.1	57.5	55.2	17.8	86.3	20.6	98.8	7.0
37	104.0	67.0	64.4	17.7	86.3	20.5	88.8	5.8
38	115.5	51.7	45.1	16.7	98.8	16.9	101.8	5.2
39	121.7	44.5	36.5	20.2	101.5	20.0	98.2	6.4
40	78.3	35.2	44.9	12.7	65.6	19.4	95.0	7.2
mean	101.4	47.6	47.7	15.8	85.5	18.5	94.9	6.0

EFFECTS OF GARLIC OIL ON SERUM AND TISSUE LIPIDS OF RATS FED DIETS WITH TWO LEVELS OF BEEF TALLOW

by

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The effect of the administration of garlic oil on serum and tissue lipids of forty male Sprague-Dawley rats fed diets with two levels of beef tallow were investigated during a 7-week feeding study. The 4 experimental groups were: low fat (4% by weight) diet, no garlic; low fat diet, garlic; high fat (19% by weight) no garlic; high fat diet, garlic. The diets were supplemented with 1% cholesterol. Garlic-fed animals received 0.01 µl of commercial garlic oil (Food Chemicals Codex grade) per gram of body weight per day. The garlic oil was suspended in 0.2 ml of corn oil and administerd by gastric intubation. Animals not receiving garlic oil were administered 0.2 ml of corn oil daily by gastric intubation. The following measurements were made: feed intake, weight gain, liver and heart weight, total serum cholesterol, HDL cholesterol, free cholesterol, liver cholesterol and serum triglycerides.

Rats fed the low fat diet had higher ($P \leq 0.01$) feed intakes but gained less ($P \leq 0.01$) weight than rats fed the high fat diet. When weight gain was adjusted linearly for feed intake in covariant analysis, the adjusted weight gain of the high fat group also was more ($P \leq 0.01$) than that of the low fat group. Supplementation with garlic did not affect feed intake or unadjusted weight gain. However, adjusted weight gain of the garlic-fed rats was lower ($P \leq 0.10$) than that of the animals not receiving garlic.

The animals fed the high fat diet had greater ($P \leq 0.01$) liver and heart weights and relative liver size than animals fed the low fat diet. No significant difference in relative heart size due to the level of fat in the diet was seen. The administration of garlic did not significantly affect liver and heart weight or relative liver size of the test animals. The

relative heart size was lower ($P \leq 0.10$) for the garlic supplemented group than for the non-supplemented rats.

Total serum cholesterol, liver cholesterol, free and ester cholesterol and serum triglycerides were higher ($P \leq 0.01$) in the high fat diet group than in the low fat diet group. No significant changes in HDL cholesterol or free/ester cholesterol ratio were observed related to fat level in the diet, but the high fat diet did produce a decrease ($P \leq 0.05$) in the HDL/total cholesterol ratio as compared to that of the low fat diet group.

Lower values were seen for total serum cholesterol ($P \leq 0.05$), ester cholesterol ($P \leq 0.10$) and serum triglycerides ($P \leq 0.10$) for animals supplemented with garlic oil than for rats receiving no garlic. Liver cholesterol was not significantly affected by the administration of garlic oil, but analysis of variance indicated an interaction between the level of fat in the diet and garlic. Liver cholesterol for the low fat diet group was lower ($P \leq 0.10$) for garlic supplemented animals than for rats receiving no garlic, but was not affected by garlic in the high fat diet group.

Results from this study show that commercial garlic oil is effective in reducing serum lipids. Further investigations are warranted to define the mechanism by which garlic affects serum lipids and serum lipoproteins.