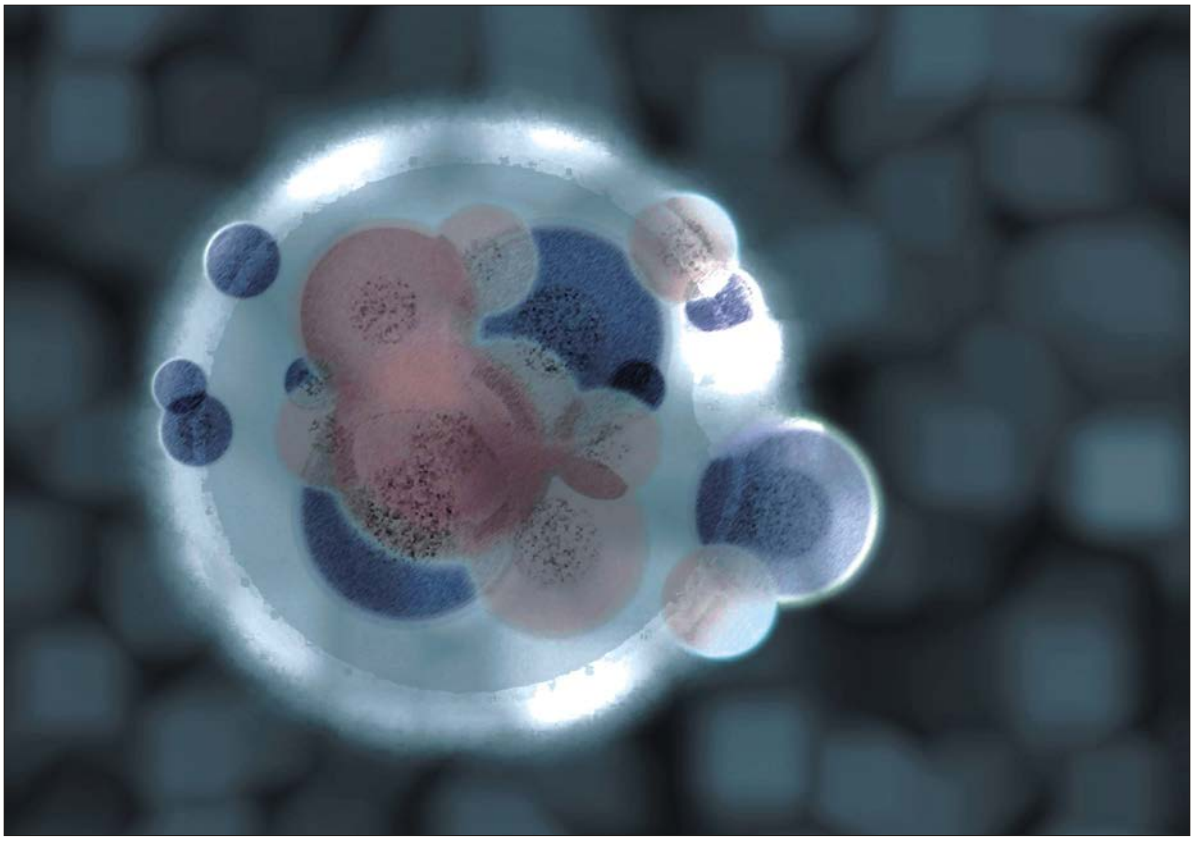

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Dietary Lycopene Has a Protective Effect on Cardiovascular Disease in New Zealand Male Rabbits

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Abstract: The aim of the present study was to determine the effect of lycopene on hypercholesterolemia in New Zealand male rabbits. Rabbits (n = 25) were divided into five groups (n = 5) and fed a normal laboratory diet (NC), a high cholesterol (HC) (0.5 g/100 g) diet, a high cholesterol diet with 42.6 ppm (HC-LYC1), 85.2 ppm (HC-LYC2) and 127.8 ppm (HC-LYC3) lycopene, respectively. Rabbits were fed their respective diets for 12 weeks. Serum was analyzed for lipids: total cholesterol (TC), (triacylglycerol) TG, LDL-C (low density lipoprotein-cholesterol), High density lipoprotein-Cholesterol (HDL-C) and apolipoproteins A1 (Apo A1) and B (Apo B). Livers were collected for measurement of HMG-CoA reductase and ACAT activities and atherosclerotic plaque formation were assessed in aorta. Results showed that rabbits fed HC-LYC1, HC-LYC2 and HC-LYC3 had significantly ($p \leq 0.05$) lower serum TC, LDL, TG, ApoB and significantly higher HDL and Apo A1 levels compared to the control-HC. HDL-C (mmol L^{-1}) in HC-LYC1, HC-LYC2 and HC-LYC3 were 2.81, 2.99 and 3.71, respectively compared to 1.93 in the control-HC group. There was a highly significant ($p \leq 0.0001$) reduction in hepatic HMG-CoA reductase levels in the groups fed the lycopene diets (HC-LYC1, HC-LYC2 and HC-LYC3) compared to the control-HC. A significant ($p \leq 0.05$) reduction in ACAT activity was seen as lycopene levels increased. The percent of atherosclerotic plaque in the aorta of the rabbits fed the control-HC diets (41.5%) was reduced to 14.8 ppm when lycopene was added to the diet at 127.8 ppm. The results of this study demonstrated that lycopene might play a significant role in the prevention of cardiovascular diseases.

Key words: Serum lipids, lycopene, HMG-CoA, ACAT, aorta plaques, atherosclerotic cardiovascular disease (ASCVD)

INTRODUCTION

Despite changes in lifestyles and the use of new pharmacologic approaches to lower cholesterol levels, cardiovascular disease (CVD) continues to be the principal cause of death. Nearly 17 million deaths are attributed to this disease (Smith *et al*., 2004). As the main cause of death in the United States (Robertson, 2001), it claimed nearly 910,614 lives in 2003 or 37.3% of all deaths that is 1 out of every 2.7 deaths (Thom *et al*., 2006). It is estimated that 71.3 million Americans have at least one form CVD (Thom *et al*., 2006).Cardiovascular disease is the collective term for all the diseases of the heart and blood vessels.

There are many risk factors associated with the increased risk for cardiovascular disease (American Heart Association, 2003). High blood cholesterol levels, high blood pressure and lack of physical activity are some risk factors that can be modified (American Heart Association, 2003). Other modifiable risk factors include smoking, obesity and diabetes (Grundy *et al*., 2000). Advancing age, gender, a family history of cardiovascular disease, reduced plasma concentrations of high-density lipoproteins and elevated plasma triglyceride concentration are also risk factors for atherosclerosis (Tribble *et al*., 2001).

Nutrition is perhaps the most significant environmental factor that has been implicated in either the development or prevention of chronic degenerative diseases (Iannelli *et al*., 2007). Nevertheless, foods that are particularly rich in natural antioxidant nutrients, such as ascorbate (Vitamin C), Vitamin E, polyphenols and

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carotenoids (Hertog *et al*., 1997; Street *et al*., 1994; Ng *et al*., 1996; Rice-Evans *et al*., 1996; Kohlmeier *et al*., 1997; Nyyssonen *et al*., 1997; Giugliano, 2000; Aviram *et al*., 2004) may have the health-protecting power to alleviate some of these chronic symptoms and diseases. The risk for heart disease is reduced significantly in populations consuming diets rich in fruits and vegetables (Willet *et al*., 1994; Mazza *et al*., 2002; Appel *et al*., 1997; Miller *et al*., 1997; Joshipura *et al*., 1999). Carotenoids are fat soluble natural pigments, which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables (Paiva and Russell, 1999). The most studied carotenoids among this diverse group of tetraterpenoids include α and β-carotene, lutein, lycopene and various xanthophylls (Mazza *et al*., 2002; Krinsky and Johnson, 2005; Stahl and Sies, 2005). Carotenoids such as Lycopene have been shown to increases Gap Junction Communication (GJC) between cells as a result of enhanced expression of connexin 43, a protein encoding a major gap junction protein (Zhang *et al*., 1992; Stock *et al*., 1998; Aust *et al*., 2003). Populations that consume foods rich in carotenoids exhibit enhanced protection against different kinds of cancer such as skin, prostate, lung and breast cancer (Thurman, 1994; Muscat and Huncharek, 1996; Pastori *et al*., 1998; Nahum *et al*., 2001; Giovannucci, 2002) and age-related macular degeneration (AMD) (Krinsky *et al*., 2003; Cardinault *et al*., 2005).

Carotenoids may protect low-density lipoproteins from oxidation, a process implicated in the development of atherosclerosis (Dugas *et al*., 1999). These beneficial effects may be due to complex interactions among carotenoids rather than to single site reactions involving individual compounds (Wahlqvist *et al*., 1994). Carotenoids, which are potent antioxidants, are capable of deactivating harmful chemical species including singlet oxygen, free radicals and lipid peroxy radicals. Various carotenoids have also been shown to stimulate the immune system and immune response (Mazza *et al*., 2002). High circulating levels of carotenoids have been thought to exhibit a protective function in the development of atherosclerosis (Klipstein-Grobusch *et al*., 2000).

Lycopene, a carotenoid which lacks provitamin A activity due to the absence of β-ionone ring (Omoni and Aluko, 2005), is present in many fruits and vegetables including watermelon, guava, apricot and papaya (Rao and Agarwal, 2000; Giovannucci, 2002; Paiva and Russell, 1999; Omoni and Aluko, 2005), but tomatoes and tomato products constitute the major source of lycopene in North American diets (Rao and Agarwal, 2000). Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases such as cancer and cardiovascular disease (Agarwal and Rao, 2000).

Lycopene is an antioxidant known to provide protection against cellular damage caused by reactive oxygen species (ROS) (Agarwal and Rao, 1998; Nguyen and Schwartz, 1998; Omoni and Aluko, 2005). ROS is a major contributor to the causation and progression of several chronic diseases including cancer and CVD (Kaliora *et al*., 2005; Omoni and Aluko, 2005; Stahl and Sies, 2005). These ROS are generated endogenously through normal metabolic activity and react and causes oxidative damage to vital biomolecules, such as lipids, proteins and DNA (Halliwell, 1994; Bandyopadhyay *et al*., 2000; Kaliora *et al*., 2005; Omoni and Aluko, 2005).

Dietary fat is a risk factor in CVD; on the other hand, studies have suggested that the consumption of dietary fat along with lycopene rich foods influences plasma levels of lycopene (Sies and Stahl, 1998; Lee *et al*., 2000; Ahuja *et al*., 2003a; Stahl and Sies, 2005). However, saturated fats are associated with the increase in blood cholesterol levels, compared to polyunsaturated fats (Carmena-Ramon *et al*., 2000; Ahuja *et al*., 2003b; Ahuja *et al*., 2006). The objective of the following study was to test the effect of selected levels of dietary lycopene on cardiovascular disease in New Zealand white rabbits.

MATERIALS AND METHODS

Animals, housing and diets: Male New Zealand White rabbits (n = 25) aged 9 week and weighing 1.91 kg were obtained from Harlan, IN. Rabbits were housed individually in standard stainless steel cages at 24°C with a 12 h light: dark cycle. Rabbits were allowed free access to food and tap water. All experiments were performed in accordance with the protocol approved by the Alabama A and M University Animal Care Committee, 2006. After 1 week of acclimatization, the rabbits were randomly assigned into 5 groups (n = 5) and fed one of the following diets for 11 weeks; rabbit chow (Harlan Teklad diets, WI) with no cholesterol (Control-NC), rabbit chow with high cholesterol (0.5 g/100 g) (Control-HC), high cholesterol diet with 42.6 ppm lycopene (HC-LYC1), high cholesterol diet with 85.2 ppm lycopene (HC-LYC2) and high cholesterol diet with 127.8 ppm lycopene (HC-LYC3) (Table 2). Lycopene was obtained from LycoMato (Israel) and experimental diets were formulated by Research Diets (Harlan Teklad diets, WI) (Table 1). Biweekly weight gain was recorded.

Table 1: Composition of control diets

Ingredients	Low control (No cholesterol)	High control (High cholesterol, HC)
Energy (kJ)	1665.20	1665.20
Protein (g)	18.00	18.00
Total fat (g)	10.00	10.00
Corn oil (g)	7.00	7.00
Lard (g)	3.00	3.00
Cholesterol (g)	0.00	0.50
Total carbohydrate (g)	59.00	59.00
Cornstarch (g)	40.00	40.00
Sucrose (g)	19.00	19.00
Salt mixture (g)	5.00	5.00
Iron (mg)	12.20	12.20
Selenium (μg)	0.90	0.90
Vitamin mixture[1] (g)	2.00	2.00
Thiamin (mg)	0.50	0.50
Vitamin E. (mg)	7.35	7.35
Fiber (g)	6.00	6.00

Mineral Composition of normal diet (per kg diet): Al, 0.5 3 mg; Ca, 6.73; Cl, 5.29; Cu, 3.43; F, 0.012 g; I, 1.55 mg; Fe, 0.122 g; Mg, 0.594; Mn, 3.64 mg; P, 3.22 g; K, 8.17 g; Se, 9.00 mg; Na, 1.50 g; S, 0.253 g; Zn, 2.98 mg, [1]Vitamin profile of normal diet (per kg diet): vitamin A, 2.75 mg as all-trans-retinyl palmitate; cholecalciferol, 25 μg; vitamin E, 73.5 mg as all-rac-alpha-tocopheryl acetate; menadione sodium bisulfate, 2 mg; biotin, 0.2 mg; cyanocobalamin, 10 μg; folic acid, 2 mg; nicotinic acid, 20 mg; riboflavin, 5 mg; calcium pentothenate, 20 mg; pyridoxine 10 mg; thiamin, 5 mg

Tissue and sample collection: Fecal pellets for each rabbit were collected daily for two weeks prior to termination of the experiment. After 12 weeks all of the rabbits were killed under CO_2 asphyxiation. Blood (after 12 h fasting) was collected by heart puncture and whole blood was immediately separated by centrifugation (4°C) and prepared for serum lipid analysis. Serum and tissue samples were stored at -20°C until used for analysis.

Analysis of fecal cholesterol: A modified method by Batta *et al.* (2002) was used to prepare fecal samples for fecal cholesterol determination. The internal standard used was 58-cholestane (Supelco, Inc Bellefont, PA). Two grams of ground sample was extracted overnight in 100 mL of petroleum ether (Fisher Scientific, Suwannee, GA) in a Soxhlet fat extractor. The ether extract was mixed with 1 mL of chloroform (Fisher Scientific, Suwannee, GA), to this mixture 0.25 g of cholestane standard (Sigma, St. louis, MO) was added and 1 μL was used for GLC analysis. Fecal cholesterol was determined by a micro-method developed by Czubayko *et al.* (1991), using a gas-liquid chromatograph (GLC). A GLC equipped with a hydrogen flame-ionization detector using a Sac-5 capillary column (Fisher Scientific, Suwannee, GA) was used. (30 m×0.25 mm d, 0.25 μm film; Supelco Inc., Bellefont, PA). Helium was used as a carrier. Temperatures were set at 230°C for the column (isothermal) and 280°C for the injector and detector temperature.

Assessment of atherosclerotic plaque: Atherosclerotic plaque areas in the aorta were assayed by the method suggested by Prasad and Kalra (1993). The analyst was blinded to the study treatment. Briefly the aortic strips were immersed in 10% buffered formalin solution (Fisher Scientific, Suwannee, GA) for 24 h and then rinsed in 70% alcohol. The tissue was later immersed in Herxheimer's solution containing Sudan IV (5 g), ethyl alcohol (70% v/v) and acetone (30% v/v) at room temperature for 15 min. The tissues were washed in distilled water. The extent of the atherosclerosis was determined and expressed as a percentage of the luminal surface that was covered by atherosclerotic plaques.

Serum lipid profile: Serum cholesterol triacylglycerol (TG) and HDL-C concentrations were determined by enzymatic methods. Serum TG concentrations were measured by hydrolyzing the triacylglycerol and measuring the released glycerol (Bucolo and David, 1973). Serum total cholesterol (TC), serum concentrations of HDL-C was determined by using a cholesterol esterase and cholesterol oxidase assay (Allain *et al.*, 1974), after removing LDL-C and VLDL cholesterol (VLDL-C) with magnesium dextran sulfate. Serum LDL concentrations were calculated according to Friedewald *et al.* (1972), which assumes that circulating VLDL consists of 80% triacylglycerol and 20% cholesterol. Apolipoprotein-(Apo) B and Apo AI were analyzed using commercially available kits (Fisher Scientific, Suwannee, GA), which use the turbidity immunoassay method of Ikeda *et al.* (1991).

HMG-CoA reductase and ACAT activities: Microsomes were prepared according to Hulcher and Oleson (1973). The resulting microsomal pellets were then dissolved in 1 mL of a homogenization buffer and analyzed for HMG-CoA and ACAT activities. The HMG-CoA reductase activities were determined as described by Shapiro *et al.* (1974) with a slight modification of using freshly prepared hepatic microsomes. The ACAT activities were determined using freshly prepared hepatic microsomes according to the method developed by Erickson *et al.* (1980) as modified by Gillies *et al.* (1986).

Statistical analysis: To determine the differences in weight gain, feed intake, fecal cholesterol excretion and serum lipids profile among the five groups (SAS, 2005) (SAS, Calabasas, Ca) was used. Results were compared for each group and significant differences ($p = 0.05$) were determined by multiple comparison tests. Fisher's LSD test was used to determine the significant differences between any two groups. Differences were considered significant at $p<0.05$.

Table 2: Experimental design

Treatment groups		Diets
No Cholesterol (NC)	Controls	Control
High Cholesterol (HC)		Control + 0.5% cholesterol
42.6 ppm Lycopene (HC-LYC1)		Control + 0.5% cholesterol + 42.6 ppm lycopene
85.2 ppm Lycopene (HC-LYC2)		Control + 0.5% cholesterol + 85.6 ppm lycopene
127.8 ppm Lycopene (HC-LYC3)		Control + 0.5% cholesterol + 127.8 ppm lycopene

NC = No Cholesterol, HC = High Cholesterol, HC-LYC = High cholesterol+ lycopene

RESULTS

Effects of dietary lycopene and cholesterol on weight gain and feed intake: Feed intake did not differ among the five different groups (data not shown). Weight gain in rabbits fed HC was significantly ($p \leq 0.05$) higher than in rabbits fed NC, HC-LYC1, HC-LYC2 and HC-LYC3 (Table 3). There were no significant ($p \leq 0.05$) differences between rabbits fed NC and HC-LYC3 (1461.80±76.72 and 1462.80±85. 60, respectively). Among the rabbits fed high cholesterol and lycopene diets, HC-LYC2 (1516.00±136.88) had a significantly ($p \leq 0.05$) higher weight gain, while the lowest weight gain was observed in HC-LYC1 (1386.60±101.99).

Table 3: Effect of dietary lycopene and a high cholesterol diet on weight gain (g) in New Zealand rabbits

Treatment groups	Weight gain (g/12 week)
Control (NC)	1461±76^{c}
Control (HC)	1673±85^{a}
HC-LYC1	1386±101^{d}
HC-LYC2	1516±136^{b}
HC-LYC3	1462±85^{c}

Values are means±SEM. ^{abcde}: Means with different superscripts are significantly different ($p \leq 0.05$) using Fisher's LSD, NC = No Cholesterol, HC = High Cholesterol, HC-LYC = High cholesterol+ lycopene-HC-LYC1-42.6 ppm; HC-LYC2-85.2 ppm; HC-LYC3- 127.8 ppm

Table 4: Fecal cholesterol excretion (mg g^{-1}) in New Zealand rabbits fed dietary lycopene

Treatment groups	Fecal cholesterol excretion
Control (NC)	0.035±0.003^{d}
Control (HC)	0.022±0.002^{e}
HC-LYC1	0.049±0.001^{c}
HC-LYC2	0.113±0.002^{b}
HC-LYC3	0.173±0.002^{a}

Values are means±SEM. ^{abcde}: Means with different superscripts are significantly different ($p \leq 0.05$) using Fisher's LSD. NC = No Cholesterol, HC = High Cholesterol, HC-LYC = High cholesterol+ lycopene-HC-LYC1-42.6 ppm; HC-LYC2-85.2 ppm; HC-LYC3-127.8 ppm

Effects of dietary lycopene and cholesterol fecal cholesterol excretion: Fecal cholesterol excretions in dietary lycopene fed groups were significantly ($p \leq 0.05$) higher than the controls (NC and HC) (Table 4). Among the dietary lycopene fed groups fecal cholesterol excretion was significantly ($p \leq 0.05$) higher in HC-LYC3 (0.173±0.002 μg g^{-1}). Among the controls, the rabbits fed HC excreted the lowest amount of cholesterol in the feces (0.02±0.002 μg g^{-1}). The rabbits fed lycopene had significantly ($p \leq 0.05$) higher cholesterol excretion with increasing lycopene levels.

Serum lipids

Serum triacylglycerol: The triacylglycerol levels were significantly ($p \leq 0.5$) different among all five groups (Table 5). The highest levels were seen in control-HC (1.88±0.012 mmol L^{-1}) and the lowest levels in HC-LYC3 (1.18±0.014 mmol L^{-1}). Among the groups fed dietary lycopene, serum triacylglycerol level was significantly ($p \leq 0.5$) higher in the group fed HC-LYC1 (1.74±0.021 mmol L^{-1}). There was a dose response relationship seen with increasing levels of lycopene and lower serum triacylglycerol levels.

Serum total cholesterol: Rabbits fed control-HC had significantly ($p \leq 0.05$) higher total serum cholesterol compared to the rabbits fed dietary lycopene; HC-LYC1, HC-LYC2 and HC-LYC3 and control NC (Table 5). There were significant differences ($p \leq 0.05$) between the two control groups fed NC and HC (4.03±0.03 and 21.83±0.28 mmol L^{-1}, respectively). Among the groups fed the high cholesterol with dietary lycopene, HC-LYC3 had significantly ($p \leq 0.05$) lower serum cholesterol (9.94±0.11 mmol L^{-1}) levels.

Serum LDL-C: There were significant ($p \leq 0.05$) differences among the controls, NC and HC (Table 5). There were significant ($p \leq 0.05$) differences among the groups fed dietary lycopene. The group fed HC-LYC3 had a significantly ($p \leq 0.05$) lower serum LDL-C level among the groups fed dietary lycopene (9.28±0.05 mmol L^{-1}). Serum-LDL-C level was approximately 42.76% lower than HC. Serum-LDL-C levels among rabbits fed dietary lycopene were significantly ($p \leq 0.05$) higher in HC-LYC3. The levels of serum LDL-C were reduced with increasing levels of lycopene.

Serum HDL-C: With increasing levels of dietary lycopene, there was a significant ($p \leq 0.05$) increase in serum HDL-C (Table 5). There were significant differences ($p \leq 0.05$) between the rabbits fed HC-LYC1 HC-LYC2 and HC-LYC3 (2.82±0.054 mmol L^{-1}, 2.99±0.014

Table 5: Effect of dietary lycopene on serum lipid concentrations in New Zealand rabbits

Treatments	Triglycerides (mmol L^{-1})	Total cholesterol (mmol L^{-1})	LDL-C (mmol L^{-1})	HDL-C (mmol L^{-1})	Apo Al (g L^{-1})	Apo B (g L^{-1})
Control (NC)	0.94±0.028^{e}	4.03±0.03^{e}	2.40±0.03^{e}	0.93±0.011^{e}	0.39±0.02^{c}	0.17±0.01^{a}
Control (HC)	1.88±0.012^{a}	21.83±0.28^{a}	16.21±0.19^{a}	1.93±0.019^{d}	0.49±0.02^{a}	0.17±0.01^{a}
HC-LYC1	1.74±0.021^{a}	18.60±0.17^{b}	14.23±0.15^{b}	2.82±0.054^{c}	0.52±0.01^{a}	0.16±0.01^{a}
HC-LYC2	1.38±0.014^{c}	14.52±0.16^{c}	12.50±0.18^{c}	2.99±0.014^{b}	0.44±0.01^{b}	0.14±0.01^{b}
HC-LYC3	1.18±0.014^{d}	9.94±0.11^{d}	9.28±0.05^{d}	3.71±0.139^{a}	0.32±0.01^{d}	0.12±0.01^{c}

Values are means±SE M. ^{abcde}: Means with different superscripts are significantly different ($p \leq 0.05$) using Fisher's LSD. NC = No Cholesterol, HC = High Cholesterol, HC-LYC = High cholesterol+ lycopene-HC-LYC1-42.6 ppm; HC-LYC2-85.2 ppm; HC-LYC3-127.8 ppm; LDL-C = Low Density Lipoprotein-Cholesterol, HDL-C = High density Lipoprotein- Cholesterol, Apo A1 = Apolipoprotein A1, Apo B = Apolipoprotein B

Table 6: Effect of dietary lycopene on HMG-CoA reductase and ACAT activities in New Zealand rabbits

Treatment groups	HMG-CoA reductase (pmol min^{-1} mg $protein^{-1}$)	ACAT (pmol min^{-1} mg $protein^{-1}$)
Control (NC)	3501.30^{b}	1102.70^{b}
Control (HC)	5200.00^{a}	2593.73^{a}
HC-LYC1	2252.30^{c}	1056.30^{c}
HC-LYC2	1505.70^{d}	750.00^{d}
HC-LYC3	1052.70^{e}	409.70^{e}

^{abcde}: Means with different superscripts are significantly different ($p \leq 0.05$) using Fisher's LSD. ACAT = acyl Coenzyme A: cholesterol o-acylytransferase, HMG-CoA = 3-hydroxy-3-methyl glutaryl Coenzyme A, HC-LYC = High cholesterol+ lycopene-HC-LYC1-42.6 ppm; HC-LYC2-85.2 ppm; HC-LYC3-127.8 ppm

and 3.71±0.139, respectively). Among the rabbits fed dietary lycopene serum HDL-C was significantly ($p \leq 0.05$) higher in HC-LYC3. Among the controls, HC had a significantly ($p \leq 0.05$) higher serum HDL-C compared to NC.

Serum Apolipoprotein AI: There were no significant ($p \leq 0.05$) differences between rabbits fed the control-HC (0.49±0.02 g L^{-1}) and the group fed HC-LYC1 (0.52±0.01 g L^{-1}). There were significant ($p \leq 0.05$) differences among HC-LYC1, HC-LYC2 and HC-LYC-3 and control NC. The levels of Apo AI increased with increasing dietary lycopene (Table 5).

Serum Apolipoprotein B: There were no significant ($p \leq 0.05$) differences among the controls; NC, HC and HC-LYC1 (Table 5). There were significant ($p \leq 0.05$) differences in the groups fed HC-LYC2, HC-LYC-3 and the controls.

HMG-CoA reductase and ACAT activities: The HMG-CoA activity in rabbits fed control-HC (5200 pmol min^{-1} mg protein) were significantly ($p \leq 0.05$) higher than control-NC and the lycopene groups (HC-LYC1, HC-LYC2 and HC-LYC-3). The greatest reduction in HMG-CoA activity was seen in HC-LYC3 (1052.7 pmol min^{-1} mg protein). This group was significantly different ($p \leq 0.05$) from HC-LYC2 and HC-LYC-3 (Table 6).

ACAT activities from the excised livers are shown in (Table 6). Rabbits fed HC-LYC3 showed the greatest reduction (409.7 pmol min^{-1} mg protein) in ACAT activities. Compared to the controls rabbits fed dietary lycopene diets (HC-LYC1, HC-LYC2 and HC-LYC-3) had significantly ($p \leq 0.05$) lower ACAT activities. Control-HC showed a significantly ($p \leq 0.05$) increased level of ACAT activity (2593.7 pmol min^{-1} mg protein) compared to control-NC (1102.7 pmol min^{-1} mg protein).

The HMG-CoA reductase and ACAT activities in the lycopene fed rabbits (HC-LYC1, HC-LYC2 and HC-LYC-3) were significantly ($p \leq 0.05$) lower than those of the control groups (NC and HC).

Table 7: Percent Atherosclerotic Plaques in New Zealand rabbits fed dietary lycopene

Treatment groups	Percent (%) plaque
Control (NC)	0.0
Control (HC)	41.5
HC-LYC1	34.5
HC-LYC2	23.4
HC-LYC3	14.8

*: Rats fed NC had no atherosclerotic plaques formations, NC = No Cholesterol, HC = High Cholesterol, HC-LYC = High cholesterol + lycopene-HC-LYC1-42.6 ppm; HC-LYC2-85.2 ppm; HC-LYC3-127.8 ppm

Atherosclerotic plaque formation: The control (NC) diet group displayed no atherosclerotic lesions in the aorta (Table 7). The HC diet group displayed 41.5% coverage of the aorta with atherosclerotic plaques. Rabbits fed HC-LYC3 showed the lowest in atherosclerotic plaque coverage with only 14.8% area covered compared to HC-LYC1 and HC-LYC2. The groups fed dietary lycopene with high cholesterol had lower reductions in atherosclerotic plaque coverage compared to control-HC. The plaque areas of the aorta were 64.3% lower in rabbits fed HC-LYC3 compared to the rabbits consuming the control- HC diet.

DISCUSSION

Evidence from scientific studies implicate the oxidation of low density lipoproteins (LDL) induced by

Reactive oxygen species (ROS) to the development of atherosclerosis, the major causative disorder leading to coronary heart disease and ischemic strokes (Rao, 2002; Blum *et al*., 2005; Prasad, 1999). The inverse relationship between disease risk and plant food consumption has been noted by a variety of dietary guidelines for Americans that encourage consumption of fruits and vegetables. Foods rich in antioxidative compounds like lycopene have the ability to reduce the risks of CVD by functioning as a potent antioxidant (Blum *et al*., 2005; Omoni and Aluko, 2005). The present study focuses on the effectiveness of lycopene in preventing CVD in hypercholesterolemia induced rabbits.

With regard to weight gain, our data suggest there were no significant ($p \leq 0.05$) differences in weight gain at the end of the 12 week study. This is consistent with results from Prasad, (1999) and Yanni *et al.* (2003). In their studies, rabbits were fed a laboratory control diet, lab diet + cholesterol or lab diet + secoisolariciresinol diglucoside (SDG), an antioxidant and aspartate and glutamate. These studies found no significant ($p \leq 0.05$) differences in the weight gains of the rabbits fed different diets.

Fecal cholesterol excretion was significantly ($p \leq 0.05$) higher with increasing levels of dietary lycopene (Table 4). The major hypothesis concerning the cholesterol-lowering effects of dietary supplements is increased fecal cholesterol and bile acid secretion (Cheung, 1998). Increased fecal excretion reduces the enterohepatic circulation of bile acids and this increases the conversion of cholesterol to bile acids (Anderson, 1987). Chan *et al.* (1999), studying the effects of jasmine Green Tea Epicatechins (GTE) in hamsters, noted that a reduction in the adsorption of dietary cholesterol was directly associated with lower serum cholesterol concentrations. The results of this study indicate that lycopene may play a role in reducing levels of serum cholesterol by increasing the cholesterol excreted in the feces.

Serum lipid profiles showed that TG, TC and LDL-C levels were significantly different ($p \leq 0.05$) among all five of the treatment groups. Rabbits fed HC diet showed the highest level of TG, TC and LDL-C, this is typical in rabbits fed a HC diet. Zhang *et al.* (2004) and Yanni *et al.* (2003) reported increased levels of TG and TC in rabbits fed a high cholesterol diet. TG, TC and LDL-C were significantly ($p \leq 0.05$) lower in rabbits fed lycopene supplemented diets (Table 5). There was a significant decrease in TG, TC and LDL-C with increasing dietary lycopene. This suggests that lycopene may play a role in the improvement of serum lipid profiles and may possibly enhance LDL-C degradation (Arab and Steck, 2000; Sesso *et al*., 2005). In contrast, not all studies show an association between lycopene and CVD (Sesso *et al*., 2003). According to Sesso *et al.* (2003), the inverse association found for tomato based products and CVD may occur through unidentified nutrients other than lycopene. It is important to note that serum lycopene levels a re higher with heat processing or when consumed with fat (Stahl and Sies, 1992; Lee *et al*., 2000; Fielding *et al*., 2005).

HDL-C, considered the good cholesterol, plays a very important role in reducing the cholesterol levels in blood and peripheral tissues (Stein and Stein, 1999; Ling *et al*., 2001). Rabbits fed lycopene (HC-LYC1, HC-LYC2 and HC-LYC3) exhibited significantly higher HDL-C levels again with increasing amounts of lycopene in the diet. Yanni *et al.* (2003) showed a marked increase in HDL-C concentrations in rabbits fed a high fat diet supplemented with aspartate and glutamate. In other studies, Lee *et al.* (2000) reported increased levels of Lycopene in rabbits fed high fat diet supplemented with flavonoids.

Apo A1 /Apo B measurements have been shown to be useful in measuring the risk of cardiovascular disease (Walldius *et al*., 2001; Liao and Ning, 2006; Lind, 2006). Apo B levels in the serum reflect the number of triglyceride-rich VLDL particles and the number of LDL particles, while Apo A1 is indicative of the number of HDL present (Walldius *et al*., 2001; Lind, 2006). Consequently, the ratio of Apo B/Apo A1 which ultimately correspond to the balance of proatherogenic and antiatherogenic lipoproteins have been reported to be a better predictor of CVD risk than TC, TG, LDL-C and HDL-C (Walldius *et al*., 2001, 2004; Yusuf *et al*., 2004; Lind, 2006).

The risk of CVD increases as the Apo B/ApoA1 ratio increases (Walldius *et al*., 2001). Evidently, the ratio of ApoA1/ApoB increased when the amount of cholesterol in the diet was increased. The ratio of ApoA1/ApoB was 2.25 in rats fed NC, but it increased to 2.83 in the HC. ApoA1/ApoB decreased with increasing dietary lycopene. This reinforces our findings which indicate the reduction of ApoA1/ApoB in rabbits fed an increased cholesterol diet with the addition of lycopene suggests an antiatherogenic effect of lycopene.

The regulation of plasma cholesterol levels involves factors that influence both the extracellular and intracellular cholesterol metabolism. The two key enzymes involved are 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and acyl-CoA: cholesterol O-acyltransferase (ACAT). HMG-CoA reductase inhibitors are very effective in lowering plasma cholesterol in most

animal species including humans (Amin *et al*., 1993). Sun and Chen (2004) and Nguyen *et al*. (2001) reported findings by Helgerud *et al*. (1981) that indicate ACAT catalyzes of the intracellular esterification of cholesterol is also involved in cholesterol absorption, hepatic VLDL-cholesterol secretion and cholesterol accumulation in the vascular wall.

This study has demonstrated a reduction in decreased plasma cholesterol and increased fecal cholesterol in rabbits fed diets supplemented with lycopene. These results suggest that, in high-cholesterol fed rabbits, intestinal cholesterol absorption was decreased by dietary lycopene. Cholesterol biosynthesis was also reduced as indicated by the decreased hepatic HMG-CoA reductase activities. Since the cholesterol intake for the HC groups was the same for all groups, except the rabbits fed NC diet, the supplementation of this compound seemed to promote a possible decrease of cholesterol uptake by tissues.

The concepts of hepatic cholesterol homeostasis and our present findings would suggest that several mechanisms underlie the metabolic effects of lycopene. Our results suggest that lycopene may reduce cholesterol biosynthesis through the inhibition of hepatic HMG-CoA reductase and ACAT, resulting in lower hepatic cholesterol level by a decreased absorption of dietary cholesterol contributing to a simultaneous increase in fecal cholesterol excretion in lycopene fed rabbits. However, administration of dietary lycopene in a high-cholesterol diet significantly decreased the activities of both hepatic HMG-CoA reductase and ACAT.

CONCLUSION

In the present study, the effects of dietary lycopene on atherosclerotic plaque formation was measured. The rabbits receiving the HC diets showed a significant ($p \leq 0.05$) increase in the amount of atherosclerotic plaques in the aorta compared to the control (NC). The HC+127.8 ppm lycopene group showed the greatest reduction in the percent of the aortic atherosclerotic plaques compared to the HC diet. The significant reductions in atherosclerotic plaque formation with the consumption of lycopene rich diets may indicate a possible role of lycopene in the protection from cardiovascular diseases.

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