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Research Article

EFFECT OF NUTS (PISTACHIO OR ALMONDS) CONSUMPTION ON LIPID PROFILE OF HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

Introduction: Nuts contain numerous beneficial nutritive and bioactive compounds like fatty acids, dietary fibbers, micronutrients, and phytochemicals that have shown favourable lipid-altering activity.

Aim: To study the effects of nut (Pistachio or Almonds) consumption on lipid profiles of hypercholesterolemic rats.

Materials and Methods: One hundred and eighty (180) adult male Sprague-Dawley rats, divided into 9 groups (20/group): G1: control; G2: hypercholesterolemic, G3-G6: hypercholesterolemic treated with: hypocholesterolemic drug, pistachio, almonds, mixture of pistachio and almonds, G7-G9 normal rats treated with pistachio (G7), almonds (8), mixture of pistachio and almonds (G9). The experiment lasted 6 weeks after treatment. Rats were sacrificed; blood was collected for biochemical analysis.

Results and Discussion: The results of this study showed that diet supplemented with pistachio and/or almond induced significant decrease in TC and LDL-C, VLDL TG and Phospholipids level, while HDL-C levels were unchanged compared to hypercholesterolemic group. The decrease being more in rats treated with mixture of both nuts. The overall result, therefore, is toward a less atherogenic lipid profile. The same was observed for MDA. This may be due to interactive or additive effects of the numerous bioactive constituents found in pistachio or almonds. Nuts are rich in several beneficial compounds, as ω-6 or ω-9 or ω-23 fatty acids which have demonstrated beneficial effects on blood cholesterol and lipoprotein profiles.

Conclusion: Results suggest that pistachio or almonds supplementation may improve blood lipids, ameliorate oxidative stress and this may be due to interactive or additive effects of the numerous bioactive constituents found in pistachio or almonds. Nuts may have beneficial applications in the prevention of cardiovascular diseases.

Keywords: Nuts, Fatty acids, CHD, CVD, Lipid profile, phytochemicals, dietary fibre**,** cholesterol.

INTRODUCTION

The World Health Organization (WHO) estimates that every year 12 million people worldwide die from cardiovascular diseases, with most of them being from the developing world (Kmietowicz, 2002). Nuts are recently recognized as "heart-healthy" foods by the U.S. Food and Drug Administration (US-FDA 2003, Kocyigit *et al.,* 2006).

Because nuts have favourable fatty acid and nutrient profiles, there is growing interest in evaluating their role in cholesterol-lowering diets. Nuts are complex plant foods that are not only rich sources of unsaturated fat (nuts are low in saturated fatty acids and high in monounsaturated and polyunsaturated fatty acids) but also contain several non-fat constituents such as plant protein, fibber, micronutrients (e.g.: copper and magnesium), vitamins as A, C, E, plant sterols, and phytochemicals that may provide additional protective effects (Kris-Etherton *et al.,* 1999 & 2001). These nutrients have shown favourable lipid-altering activity (Vorster *et al.,* 2003). Nuts such as almonds and pistachios are rich in several beneficial compounds, such as ω-9 fatty acids, which have demonstrated beneficial effects on blood cholesterol and lipoprotein profiles (Brown 2003). It has been proposed that the bioactive compounds in nuts may help lower the risk factors of CVD by improving endothelial function (Ros 2009), blood pressure (Estruch *et al.,* 2006) and the serum lipid profile (Griel & Kris-Etherton 2006) in addition to lowering oxidative stress (Jenkins *et al.,* 2006 & 2002) and inflammation (Jiang 2006).

There are controversial results about the effects of pistachio nut and/or almonds consumption on the lipid profile of patients with hypercholesterolaemia. There is limited information about the antioxidant effects of nuts and pistachios (Matthäus and Ozcan, 2006).

MATERIAL AND METHODS

Approval of the experimental protocol had been taken from the research ethics committee of General Organization of Teaching Hospitals & Institutes (GOTHI), Cairo, Egypt.

Animals and diet

Pistachio and almonds were purchased from local markets of Cairo, Egypt. They were analysed for their nutritive value according to AOAC (2010). They were analysed for their fatty acid profile using GC mass.

Fatty acid Profile

a) Lipid extraction was conducted using method of AOAC 2000. Separation of fatty acids (saponification, preparation of diazomethane, then methylation) was carried out using method of Vogel 1975.

b) Identification and determination of fatty acids was conducted using gas liquid chromatography (GLC, GC trace GC ULTRA) according to Farag *et al.,* 1986. The gas chromatographic analysis was performed on a GC trace GC ULTRA equipped with an FID fitted with a column (30 m) packed with 70% cyanopropyl polysilphenylene siloxane. The carrier gas was N_2 with a flow rate of 1.5 ml/min. The column was run isothermally at 195 ^{0}C and the injector and detector were at 220 ^{0}C. The fatty acids were identified by the retention time by comparing with standards. Peak area was measured by using a computing integrator (PU 4810, Philips).

Experimental Design

One hundred and eighty (180) adult male Sprague-Dawley rats weighing 210±20 g, 3 months old were housed individually in stainless steel mesh cages. They were fed on standard diet for 10 days before experiments began (Adaptation period). The control diet was prepared according to Reeves *et al.,* (1993), and National Research Council (NRC) Committee on Animal Nutrition, (1978). The diets were prepared every week in the laboratory. The water and diets were given ad libitum. Induction of hypercholesterolemia was carried out on 6 groups (G2-6; 100) by addition of cholesterol (2 %) + 0.25 % bile salts to the basal diet for 4 weeks. The animals (180) were divided into 9 groups (20 rat/group) as follows: Group 1 (Control group, -ve control): rats fed on basal diets; Group 2: Hypercholesterolemic rats, (+ve control); Group 3: Hypercholesterolemic rats fed on basal diet supplemented with hypocholesterolemic drugs (Statin); Group 4: Hypercholesterolemic rats fed on basal diet supplemented with pistachio 2%; Group 5: Hypercholesterolemic rats fed on basal diet supplemented with almonds 2%; Group 6: Hypercholesterolemic rats fed on basal diet

supplemented with equal mixture of pistachio and almonds; Group 7: normal rats fed on basal diet supplemented with pistachio 2%; Group 8: normal rats fed on basal diet supplemented with almonds 2%; Group 9: normal rats fed on basal diet supplemented with equal mixture of pistachio and almonds. Diets and rats weight were done once/week.

At the end of the experimental period (6 weeks after treatment), rats were fasted over night before sacrificing, blood was collected, centrifuged; serum was stored at - 80 ^{0}C until analysis. Part of the blood is collected on tubes coated with EDTA. Some minerals as Mg, Cu, Fe etc… and some vitamins as A, C and E were determined in nuts.

Analytical Methods

The serum total cholesterol (TC) and serum high density lipoprotein cholesterol (HDL-C) level was determined using colorimetric enzymatic kits (SGM Italia, Rome, Italy), according to the method described by Allian *et al.,* (1974) and Lopes-Virella *et al*., (1977) respectively. The serum low density lipoprotein cholesterol (LDL-C) level was determined using colorimetric enzymatic kits (SGM Italia, Rome, Italy), according to the method described by Fruchart *et al*., (1982) and Levy *et al.,* (1981).The serum triacylglycerol (TG) level was determined using colorimetric enzymatic kits (SGM Italia, Rome, Italy), according to the method described by Bucolo *et al*., (1973). Very low density lipoprotein cholesterol (VLDL-C) level was calculated using the following equation: VLDL-C= TC–(HDL-C+LDL-C). The plasma malonedialdehyde (MDA) level was determined according to the method described by Draper and Hadley (1990). Amino acids (arginine and lysine) were determined using amino acid analyzer, AAA, Sykum using its instruction cited in the catalogue. Minerals were determined using atomic absorption according to AOAC 2010. Vitamins were determined using HPLC according to AOAC 2010.

Statistical Analysis

All results were expressed as the mean ± SE. Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 11.0, Chicago, IL, USA). The data were analyzed by one-way analysis of variance (ANOVA). To compare the difference among the groups, post hoc testing was performed by the Tukey test. Pearson's correlation analysis was used to determine the correlation among the parameters assessed. The p- value < 0.05 was considered statistically significant (Dawson and Trapp, 2001).

RESULTS

Table (I-a) reveal nutritive value, mineral and vitamin content of pistachio and almonds nuts. The kernels are a rich source of oil (>50.5%). It contains protein (>18.6), carbohydrate (> 16.87%) and dietary fibre (>10.0%). Pistachio nut also contains high amounts of K and P, and various amounts of Ca, Mg and Fe. The caloric value of the pistachio nuts and almonds was 633.33, 613.3 Kcal/100 gm respectively of the edible parts which is in agreement with Breuer 1993, Food Composition Tables for Egypt 2006.

Table 1-a: Chemical analysis (nutritive value) of pistachio and almonds/100gm.

	Protein	Ash	Crude fibre	Mois-ture	Carbo-hydrate	Dietary fibre	Calories Kcal/100 gm	Minerals (mg/100gm)						Vitamins		
			gm/100 gm					Ca	Fe	Na	K	Cu	Mg	C	A	E
								mg/100 gm						mg	IU	mg
Pistachio	20.71	2.14	1.43	5.18	16.87	12.5	633.33	107.14	3.93	270	1039.29	1.43	160.0	5.71	42.14	26.07
Almonds	18.6	2.7	2.5	4.6	21.1	10.0	613.3	215	1.8	7	793	0.13	205	ND	21	26

ND: Not detected

Table (I-b) reveal fat content and amount of mono-, polyunsaturated and saturated fatty acids of pistachio nuts and almonds. Also it shows their fatty acid composition. Pistachio nuts and almonds are highly nutritious foods, its fat content as high as 53.67; 50.5 %. Table (I-b) reveals that the mean fatty acid composition of the pistachio nut and almonds is 60.51, 65.41% oleic acid; 27.69, 17.42% linoleic acid, 10% palmitic acid which agrees to somewhat with (Gamlyi and Hayoglu 2007). It also reveals that pistachio and almonds are rich in unsaturated fatty acids which represent 88.71, 83.7% of fat respectively.

Table 1-b: Fat content and % MUSFA, PUSFA, SFA, fatty acid composition and arginine & lysine content of pistachio nuts & almonds

	Fat (gm/100 gm)					Relative % of fat						Amino acid		
	Total	MUSFA	PUSFA	SFA	SFA USFA	Myristic	Palmitic	Palmitoleic	Stearic	Oleic (ω 9)	Linoleic (ω 6)	Arginine	Lysine	Arg: Lys
Pistachio	53.67	32.75	14.86	5.77	5.77: 47.61	0.75	10	0.51	----	60.51	27.69	2.21	1.19	1.86:1
Almonds	50.5	33.47	8.8	8.22	8.23: 42.27	6.06	9.33	0.88	0.9	65.41	17.42	2.56	0.65	3.94:1

MUSFA: Monounsaturated Fatty Acids; PUSFA: Polyunsaturated Fatty Acids; SFA: Saturated Fatty Acids. ND: Not detected

Table (II) reveals that hypercholesterolemic rats showed significantly decreased body weight when compared with normal control group. At the end of the experiment, the mean levels of body weight of G3-G6 rats were significantly lower than control group but significantly higher than hypercholesterolemic rats but in control+pistachio, control + almonds, control+mixture groups (G7-G9), it shows no significant change in body weight compared to normal control group, but it show significantly higher body weight when compared with hypercholesterolemic rats or treated groups (G3-G6).

Table (III) reveals that cholesterol, LDL-C, VLDL-C, triacylglycerol, phospholipids and malondialdehyde levels were significantly higher (P< 0.001) in hypercholesterolemic-induced rat group (G2). Pistachio and/or almonds consumption significantly decreased cholesterol, LDL-C, VLDL-C, phospholipids TG and MDA levels (P < 0.001, respectively). The results agree with Griel and Kris-Etherton (2006), Phung *et al.,* (2009), Sheridan *et al.,* (2007), Kocyigit *et al.,* (2006) and Aksoy *et al.,* (2007).

Table 2: Initial, Final body weight (IBW, FBW), weight gain, Lengh (cm) and body mass index (BMI) of groups under study in comparision with normal and hypercholesterolemic rats

	IBW	FBW	WG	Length	BMI
		gm		cm	
G1	226.30±1.93	300.00±2.51	73.70±1.95	20.00±0.31	7.54±0.20
G2	223.30±3.17	186.10±3.55 [a]	-37.20±3.29 [a]	20.10±0.23	4.61±0.09 [a]
G3	227.30±1.64	277.10±1.41 [a,b]	49.80±2.54 [a,b]	20.25±0.13	6.76±0.08
G4	228.00±1.34	278.50±1.19 [a,b]	50.50±1.26 [a,b]	20.11±0.29	6.92±0.18
G5	228.40±1.89	279.30±1.08 [a,b]	50.90±2.75 [a,b]	20.10±0.30	6.95±0.20
G6	228.90±0.96	280.90±1.91 [a,b]	52.00±2.10 [a,b]	20.05±0.22	7.00±0.13
G7	223.90±2.76	295.90±1.36 [a,b,c,d]	72.00±2.31 [a,b,c,d]	20.10±0.23	7.35±0.17 [a,b,c,d]
G8	227.60±0.99	299.30±1.39 [a,b,c,e]	71.70±1.35 [a,b,c,d]	20.25±0.13	7.31±0.09 [a,b,c,e]
G9	228.00±1.34	299.60±1.25 [a,b,c,f]	71.60±1.13 [a,b,c,f]	20.11±0.29	7.45±0.20 [a,b,c,f]

G1: Normal control; G2: Hypercholesterolemic; G3-G6: hypercholesterolemic rats treated with: (G3); pistachio nuts (G4); almonds (G5); mixture of pistachio nuts and almonds (G6); G7: Normal+Pistachio; G8: Normal+Almonds; G9: Normal+ mixture of pistachio nuts and almonds.

a: significant from G1; b: significant from G2; c: significant from G3; d: significant from G4; e: significant from G5. Significant at P< 0.001

Table 3: Effect of nuts (pistachio and/or almonds) consumption on lipid profile of hypercholesterolemic rats.

	S. Cholesterol	S. HDL-C	S. LDL-C	S. VLDL-C	TG	Phos.Lip	Chol HDL-C	HDL-C LDL-C	MDA
				mg/dl					(µmol/L)
G1	77.94±1.15	40.39±0.35	24.06±0.58	13.50±1.05	60.42±1.87	487.89±2.20	1.93±0.03	1.69±0.04	70.92±2.38
G2	202.46±2.42 [a]	41.62±0.64	132.90±1.75 [a]	27.94±2.37 [a]	100.81±3.51 [a]	847.29±11.07 [a]	4.87±0.09 [a]	0.31±0.01 [a]	124.14±2.72 [a]
G3	99.86±2.99 [a,b]	41.80±0.92	39.58±2.68 [a,b]	18.48±0.54 [a,b]	80.45±1.59 [a,b]	600.37±7.82 [a,b]	2.40±0.08 [a,b]	1.07±0.05 [a,b]	79.92±1.56 [a,b]
G4	106.89±2.99 [a,b]	41.57±0.92	46.91±2.68 [a,b,c]	18.40±0.54 [a,b]	78.26±1.59 [a,b]	698.57±7.82 [a,b,c]	2.58±0.08 [a,b]	0.91±0.05 [a,b,c]	90.86±1.56 [a,b,c]
G5	107.06±1.67 [a,b,c]	42.77±0.48 [a]	45.21±1.16 [a,b,c]	19.08±0.97 [a,b]	80.50±1.36 [a,b]	697.06±7.55 [a,b,c]	2.51±0.05 [a,b]	0.95±0.03 [a,b,c]	90.73±1.77 [a,b,c]
G6	100.52±1.98 [a,b]	42.37±0.50 [a]	42.40±0.89 [a,b]	15.76±1.52 [b]	74.55±1.55 [a,b,c,d,e]	603.10±7.40 [a,b,d,e]	2.37±0.05 [a,b]	1.00±0.02 [a,b]	85.56±1.54 [a,b,c,d,e]
G7	72.49±0.81 [a,b,d]	39.37±0.59 [a,b,d]	23.84±0.48 [a,b,d]	9.27±0.45 [a,b,d]	66.29±1.37 [a,b,d]	478.34±9.12 [b,d]	2.43±0.04 [a,b]	0.95±0.03 [a,b]	68.97±1.51 [b,d]
G8	73.39±1.25 [a,b,e]	39.90±0.83 [a,b,e]	24.08±0.48 [a,b,e]	9.41±0.14 [a,b,e]	70.45±2.43 [a,b,e]	479.37±8.81 [b,e]	2.37±0.02 [a,b]	0.99±0.01 [a,b]	69.92±1.15 [b,e]
G9	66.94±1.50 [a,b,f]	39.89±0.87 [a,b,f]	18.21±0.81 [a,b,f]	8.84±0.16 [a,b,f]	55.56±1.56 [b,f]	448.57±7.82 [b,f]	2.33±0.06 [a,b]	1.09±0.05 [a,b]	65.86±1.56 [b,f]

G1: Normal control; G2: Hypercholesterolemic; G3-G6: hypercholesterolemic rats treated with: (G3); pistachio nuts (G4); almonds (G5); mixture of pistachio nuts and almonds (G6); G7: Normal+Pistachio; G8: Normal+Almonds; G9: Normal+ mixture of pistachio nuts and almonds.

a: significant from G1; b: significant from G2; c: significant from G3; d: significant from G4; e: significant from G5; f: significant from G6; Significant at P< 0.001

Mechanisms for the Action of Statins

Statins act by competitively inhibiting HMG-CoA reductase, the first committed enzyme of the HMG-CoA reductase pathway. Because statins are similar to HMG-CoA on a molecular level they take the place of HMG-CoA in the enzyme and reduce the rate by which it is able to produce mevalonate, the next molecule in the cascade that eventually produces cholesterol (synthesis of cholesterol in the liver), as well as a number of other compounds. They alter the conformation of the enzyme when they bind to its active site. This prevents HMG-CoA reductase from attaining a functional structure. The change in conformation at the active site makes these drugs very effective and specific. Binding of statins to HMGCoA reductase is reversible, and their affinity for the enzyme is in the nanomolar range, as compared to the natural substrate, which has micromolar affinity (Corsini *et al.,* 1999).This ultimately reduces cholesterol via several mechanisms.This is significant because most circulating cholesterol comes from internal manufacture rather than the diet. When the liver can no longer produce cholesterol, levels of cholesterol in the blood will fall. Cholesterol synthesis appears to occur mostly at night (Miettinen 1982). Efficacy on triglyceride reduction parallels LDL cholesterol reduction (Stein *et al.,* 1998).

In rabbits, Liver cells sense the reduced levels of liver cholesterol and seek to compensate by synthesizing LDL receptors to draw cholesterol out of the circulation (Ma *et al.,* 1986). This is accomplished via protease enzymes that cleave a protein called "membrane-bound sterol regulatory element binding protein", which migrates to the nucleus and causes increased production of various other proteins and enzymes, including the LDL receptor. The LDL receptor then relocates to the liver cell membrane and binds to passing LDL and VLDL particles. LDL and VLDL are drawn out of circulation into the liver where the cholesterol is reprocessed into bile salts. These are excreted, and subsequently recycled mostly by an internal bile salt circulation (Ma *et al.,* 1986).

Statins inhibit hepatic syntesis of apolipoprotein B-100, determining a reduction of the synthesis and secretion of triglyceride rich lipoproteins and an increase of receptors production for apolipoproteins B/E (Gaw *et al.,* 1993). Statins have a modest effect on HDL increase, and no influence on lipoprotein(s) concentration (Kostner *et al.,* 1989).

DISCUSSION

Although nuts are nutrient that has high fat content, there is a fear of increased energy intake but surprisingly nut consumption has been associated with lower or no change in BMI with no fear of weight gain (Bes-Rastrollo *et al.,* 2009; Mattes *et al.,* 2008). Very few studies have specifically examined the effects of nuts on body weight (Fraser *et al.,* 2002). In our study there was no significant change in body weight of normal rats consuming nuts which agrees with Edwards *et al.,* 1999 (in humans study using pistachio or almonds) and agree

also with Spiller *et al.,* 1992, Jenkins *et al.,* 2002 for almonds, and disagree with Lovejoy *et al.,* 2002 for almonds where a significant increase in body weight was found in their study. Many of the supplementation studies that have examined the effects of nuts on lipid profiles have not found negative effects on body weight (Jenkins *et al.,* 2002; and Edwards *et al.,* 1999). The non significant change in body weight may be due to some degree of mal absorption of energy in nuts (Fraser *et al.,* 2002). In this study a significant decrease in body weight of hypercholesterolemic rats were observed, but after supplementation of the diet with nuts the weight begun to increase, still significantly lower than normal control but body weight gain of rats after the supplementation closely near body weight gain of normal group in this period (unpresented data). In our study no direct correlation between weight gain and nut consumption were found which agree with Sabate´ 2003.

In this study, TC and TG levels were significantly increased in the hyperlipidaemic group compared with the control group. Also the results of this study showed that diet supplemented with pistachio and/or almond induced significant decrease in TC and LDL-C, VLDL level, while HDL-C levels were unchanged (the reduction in TC being attributed to changes in LDL-C) of hypercholesterolemic rats compared to control group consuming normal control diets. Clinical and epidemiological studies have reported the beneficial effects of tree nuts and peanuts on serum lipid levels (Aksoy *et al.,* 2007; Eme kli-Alturfan *et al.,* 2007). On the other hand, there are controversial results about the effects of pistachio nut and/or almonds consumption on the lipid profile of patients with hypercholesterolaemia. Our results disagree with Sheridan *et al.,* 2007 who studied the effects of 15 % of the daily caloric intake in the form of pistachio nuts on the lipid profiles of free-living human subjects with primary and moderate hypercholesterolaemia and found no significant differences in TC and TG levels and agree with Edwards *et al.,* 1999 who reported decreased TG and TC levels in patients with hypercholesterolaemia; and agree with Kocyigit *et al.*, 2006 who observed non-significant decreases in TG levels in healthy volunteers.

The overall result, therefore, is toward a less atherogenic lipid profile. A 1% drop in serum cholesterol reduces the risk for CHD by 2% (Jain *et al.,* 2007). Kinosian *et al.,* (1995) and Natarajan *et al.* 2003 have reported that changes in ratios of TC/HDL-C and LDL-C/HDL-C are better predictors of CHD risk reduction than changes in levels (Panagiotakos *et al.,* 2003). The dietary intervention did alter these ratios in a cardio-protective direction. In our study the beneficial effects of pistachio and/or almonds consumption over the six-week intervention period were modest (the decrease reach > 66% in comparison with G2, it is even better >75% on combination of both nuts), so it is possible that the cumulative effect of long term consumption could prove cardio-protective and help lower coronary artery disease.

The primary sources of fat in the treated groups were from pistachio or almonds. This effect on lipids may be due to pistachio or almonds type of fats or to other factors as the influence of other substances contained in the nuts or in the diet.

Nuts are low in saturated fatty acids (SFA: 5.77 gm/100 gm for pistachio; and 8.22 gm/100 gm for almonds) and high in unsaturated fatty acids (USFA: 47.61 gm/100 gm for pistachio; and 42.27 gm/100 gm for almonds). The predominant type of unsaturated fatty acid in most nuts is MUFA (32.75 gm/100 gm for pistachio; and 33.47 gm/100 gm for almond). USFA (MUFA and PUFA) contribute 88.71 (pistachio): 83.7 (almonds) % of the energy from fat.

There are persuasive evidences that dietary substitution of monounsaturated fatty acids (MUFA) or ω -6 polyunsaturated fatty acids (PUFA) for SFA lowers blood cholesterol and may have beneficial effects on inflammation, thrombosis, and vascular reactivity. MUFA may have an advantage over PUFA because enrichment of lipoprotein lipids with MUFA increases their resistance to oxidation. Intake of unsaturated fatty acids with nuts is intrinsically cardio protective (Kris-Etherton, 1999; and Kris-Etherton *et al.,* 2001).

Cholesterol levels in the body result from two sources: absorption from the gastrointestinal tract and endogenous *de novo* synthesis. The reduction in the values of lipid profile levels may be due to inhibition of hepatic cholesterol synthesis, or the redistribution of cholesterol from plasma to the liver by the cholesterol metabolizing enzyme systems in the liver or the control of lipids utilization.

Nuts such as almonds and pistachios are rich in several beneficial compounds, as ω-6 or ω-9 or ω-23 fatty acids which have demonstrated beneficial effects on blood cholesterol and lipoprotein profiles (Sabate´ 2003). Also, it has been shown to elicit cardio protective effects. The highly unsaturated ω-23 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are primarily responsible for this effect (Stone *et al.,* 1996). Metabolic studies have shown that consumption of n-6 PUFA lowers circulating cholesterol level (Ramachandran *et al.,* 2003).

Nuts are complex plant foods that are not only rich sources of unsaturated fat but also contain several non-fat constituents such as plant protein, fibber, micronutrients (e.g.: copper and magnesium), vitamins as A, C, E, plant sterols, and phytochemicals that may provide additional protective effects (Kris-Etherton 1999; and Kris-Etherton *et al.,* 2001). These nutrients have shown favourable lipid-altering activity (Vorster *et al.,* 2003).

The first possibility may be the source of protein that was added to the diet. We used casein in the control diet which supplied about 16 g of milk protein/100 gm diet to replace the majority of the protein provided by pistachio or almonds. There is significant literature indicating that amino acid profiles, including the arginine: lysine ratio (pistachio: 2.21/1.19; almonds: 2.56/0.65) in nuts, have beneficial effects on blood lipids when compared with animal proteins (Jenkins *et al.,* 1989). Further, protein in nuts has an arginine-rich amino acid profile that is thought to be protective (Kritchevsky *e al.,* 1982). Arginine, one of the most abundant amino acid found in nut proteins, may account for the hypocholesterolemic effect observed in animal studies (Kurowska and Carroll 1994) or in human intervention trials (Maxwell *et al.,* 2000). In addition, pistachios are relatively high in the semi essential amino acid arginine (2.21g/21.4g protein), which appears to maintain flexible arteries and to enhance blood flow by boosting nitric oxide, a compound that relaxes blood vessels. There is significant literature indicating that amino acid profiles, including the favorable arginine: lysine ratio in nuts, have beneficial effects on blood lipids when compared with animal proteins (Kurowski and Carroll 1994 and Jenkins *et al.,* 1989).

A second possible explanation may be the contribution of the dietary fibers supplied by the pistachio or almonds (pistachio: 12.5 g/100g, of which ≈ 25 % is soluble fibre; almonds: 10.0 g/100g, of which ≈ 10% is soluble fibre respectively, provide ≈10% of the DRI). Soluble fibre has been shown to reduce total and LDL-cholesterol concentrations and improve glycemic control (Anderson *et al.,* 1994 and Brown *et al.,* 1999). They concluded that 2–10 g/d of soluble fibre was associated with small but significant decreases in TC.

A third possible explanation is the presence of lipid-altering phytochemicals such as plant sterols and saponins that are found in almonds (Farquhar 1996; and Oakenfull 1996). Also pistachios contain significant amounts of phytosterols and other phytochemical compounds such as polyphenols and ellagic acid. The major phytosterol component is β-sitosterol, which is one of several plant sterols implicated in cholesterol lowering (Jones *et al.,* 1997). Studies showed that 2 g of plant sterols/d significantly reduces cholesterol absorption, which in turn decreases plasma TC and LDL-C concentrations (Farquhar 1996; Oakenfull 1996 and Vorster *et al.,* 2003).

Almonds contain a variety of phenolic compounds, localized principally in their skin, including flavonols, flavanols, flavanones, anthocyanins procyanidins (B_2 and B_3), and phenolic acids (caffeic acid, ferulic acid, p-coumaric acid, protocatechuic acid, vanillic acid) (Amarowicz *et al.,* 2005 and Wijeratne *et al.,* 2006). Almond flavonols and flavanols have been shown to be bioavailable and contribute to the antioxidant protection against LDL-C oxidation in vitro and in vivo (Jenkins *et al.*, 2002 and Chen *et al.,* 2005).

A Fourth possible explanation is that almost all nuts are good sources of minerals as magnesium, copper. Magnesium (Mg) level in nuts (pistachio and almonds), provide 8–10% of the DRI for this essential mineral in a 25 g. RDI of Mg is 360-400 mg/day for adult human (RDIs 1997) Magnesium is important since low magnesium status can contribute to myocardial infarction, and possibly hypertension. Magnesium is also critical to enzyme function. Copper (Cu) in nuts (pistachio, almonds), ≈50, 5% of the DRI for copper (700 µg/day) (RDIs 1997) respectively and therefore nuts can be a significant source of this essential mineral (Allen *et al.,* 1977). Copper plays a key role in hematopoiesis and diets low in copper has been associated with adverse changes in lipids, glucose tolerance, blood pressure, and electrocardiograms (Klevay 1993).

A Fifth possible explanation is nuts content of Vitamin E. Vitamin E in high doses (> 100 IU/d) has been shown to reduce the risk of coronary heart disease (Rimm and Stampfer1997). This cardio-protective effect appears to be due to vitamin E-induced inhibition of LDL oxidation [vitamin E is transported in the LDL particle (Steinberg and Lewis 1997)], a key step in the atherogenic process. Nuts are a rich source of vitamin E, although the quantities obtained from typical nut consumption are far less than the amounts shown to have beneficial effects on coronary heart disease. Nonetheless, nut consumption is still an effective means of increasing vitamin E intake. RDI of vitamin E is 15 mg/day for adult human. Almonds in particular are especially rich in many tocopherols, including α-tocopherol, the most active form of vitamin E, which has also shown potent anti-atherogenic effects (Food and Nutrition Board, 2004, Jenkins *et al.*, 2002 and Chen *et al.,* 2005).

It is apparent that fibre, vitamin E, arginine, phytosterols, and phenolic components from realistic amounts of nuts are not sufficient to exert individual hypocholesterolemic effects. In essence, it is possible that there are multiple small effects that contribute, and these are mediated by more than the lipid-lowering fatty acid composition.

A sixth possible explanation that might explain the cholesterol-lowering effects of pistachios are through Stearoyl-CoA desaturase (SCD) and cholesteryl ester transfer protein (CETP). Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme that catalyzes the synthesis of MUFAs; 18:1 and 16:1 from SFAs; 18:0 and 16:0 and plays an important role in cholesterol, triacylglycerol, and lipoprotein metabolism. Stearoyl-CoA desaturase (SCD) plays an important role in lipid metabolism by catalyzing the synthesis of MUFAs, mainly () 18:1 and () 16:1, from SFAs. The ratio of SFAs to MUFAs in plasma reflects the membrane phospholipids composition, and increases in this ratio have been implicated in diseases such as CVD, obesity, and diabetes (Ntambi and Miyazaki 2004) so consumption of nuts that contains high levels of unsaturated fats resulted in a significantly lower ratio of 16:1/16:0. The direct correlations between change in SCD activity and lipids and lipoproteins suggest that SCD activity may contribute to the lipid-lowering effects of pistachios. Chole steryl ester transfer protein (CETP) is a plasma protein that plays a key role in reverse cholesterol transport by transferring cholesteryl esters (CEs) from HDL particles to LDL and VLDL particles in exchange for triacylglycerols. CETP may be antiatherogenic in that it increases the rate of reverse cholesterol transport, but it may be proatherogenic in that it transports CE from HDL, which is protective, to VLDL and LDL, which are atherogenic (Cuchel and Rader 2007). Studies in humans have shown that the intakes of SFAs (Schwab *et al.,* 1996) and trans fatty acids (Van *et al.,* 1995) increase CETP, whereas the intake of MUFAs decreases (Jansen *et al.,* 2000) and the intake of PUFAs decreases (Bagdade *et al.,* 1992) or has no effect on CETP (Thomas *et al.,* 2004).

Oxidative stress (disruption of the balance between oxidative and antioxidative processes), plays an important role in the pathogenesis of atherosclerosis (Steinberg *et al*., 1989). A cholesterol rich diet results in increased lipid peroxidation by the induction of free radical production, followed by hypercholesterolemia, a major risk factor for atherosclerosis. It has been reported that hypercholesterolemic atherosclerosis is associated with an increase in tissue concentration of lipid peroxidation products, malondialdehyde and conjugated dienes (Lorgeril *et al*., 1994).

Scientists have concluded that overproduction of reactive oxygen species (ROS) (oxidative stress) plays a pivotal role in the oxidation of LDL molecules, which get accumulated in the layers of blood vessels. Lipid oxidation due to generation of ROS is considered as an important factor in the initiation and progression of several diseases (Fasoriyo and Adegoke 2006). The amount of lipid peroxide was measured by MDA assay, which is considered as indirect measure of the formation of lipid peroxides free radicals.

In the present study TBARS levels significantly increased in the hyperlipidaemic group. Decreased antioxidant levels are possibly due to their increased utilization combating excessive plasma oxidative stress in hypercholesterolaemic rats. Consequently, decreased TAA in the hyperlipidaemic group might be responsible for the increased peroxidation of the membrane lipids in this group since increased peroxidation of membrane lipids causes reduction in the activity of antioxidative enzymes. Disturbed balance between oxidants and antioxidants due to hyperlipidaemia has been shown before (Emekli-Alturfan *et al.,* 2008). On the other hand, there is an increasing but inconclusive body of evidence suggesting that nuts improve antioxidant levels (Kocyigit *et al.,* 2006; Gentile *et al.,* 2007; Emekli-Alturfan *et al.,* 2008). Consequently, in the present study pistachio and/or almonds supplementation in the hyperlipidaemic group significantly decreased TBARS levels when compared with the untreaed hyperlipidaemic group.

The antioxidant effects of pistachio against oxidative damage might originate from phytochemicals in its content that have strong free radical scavenging ability (Tapiero *et al.,* 2002; Tokusoglu *et al.,* 2005; Kocyigit *et al.,* 2006). Polyphenols, including flavonoids, can exert their antioxidant activity by inhibiting the activities of enzymes, including lipooxygenase and cyclooxygenase, by chelating metal ions, and, most importantly, by scavenging free radicals. Generally, polyphenols are potent free radical scavengers because phenolic groups are excellent nucleophiles (Tapiero et al., 2002). Moreover, it may be assumed that polyphenols in pistachio or almonds reinforce the antioxidant system. These results suggest that pistachio and/or almonds could be a useful compound to control hypercholesterolaemia by both improving the lipid profile and modulating oxidative stress. This modified balance between the antioxidative enzymes might be able to remove superoxides more efficiently (Tapiero *et al.,* 2002; Tokusoglu *et al.,* 2005; Kocyigit *et al.,* 2006).

Jenkins *et al.,* 2008 predicted that the higher intake of vitamin E, MUFA, and phenolic constituents with almond consumption, and the interactions between these nutrients, would increase the status of vitamin E and decrease the level of lipid peroxidation, specifically reducing the biomarkers of oxidative damage, serum MDA.

CONCLUSION

This study supports the benefits of a diet supplying a reasonable amount of fat as monounsaturated fat, while low in saturated fat, for control of plasma cholesterol. Results suggest that pistachio or almonds supplementation may improve blood lipids, ameliorate oxidative stress and this may be due to interactive or additive effects of the numerous bioactive constituents found in pistachio or almonds. Nuts may have beneficial applications in the prevention of cardiovascular diseases.

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