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ORIGINAL CONTRIBUTION

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Peanuts improve blood glutathione, HDL-cholesterol level and change tissue factor activity in rats fed a high-cholesterol diet

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Abbreviations TF: Tissue factor · GSH: Glutathione · TBARS: Thiobarbituric acid reactive substances · TL: Total lipid · TC: Total cholesterol · HDL-C: HDL-cholesterol · LDL-C: LDL-cholesterol · TG: Triacylglycerols · PT: Protrombin time · APTT: Activated partial thromboplastin time · SFA: Saturated fatty acids · AOP: Antioxidant potential

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Abstract *Background* The inverse association of peanut consumption and risk markers of CHD (lipids) has been reported however health professionals are still concerned whether hyperlipidemic subjects advised to eat peanuts will have increased serum lipid levels. Tissue factor (TF), the major regulator of normal haemostasis and thrombosis, plays a critical role in haemostasis in all tissues. *Aim of the study* To investigate the effects of peanut consumption on lipid profile, blood Glutathione (GSH), thiobarbituric acid reactive substances (TBARS), haematologic parameters and TF activities in rats fed a high-cholesterol diet. *Methods* 32 Wistar Albino rats were divided into 4 groups of 8 rats each: 1-Control 2-Control+peanut 3-Hyperlipidemic and 4-Hyperlipidemic+peanut group. At the end of 12 weeks, blood samples were used to evaluate lipid profile, haemostatic parameters, GSH, TBARS and tissue samples were used for the determination of TF activities. *Results* Peanut consumption increased blood GSH both in the control and hyperlipidemic groups; increased HDL-cholesterol and decreased TBARS in the hyperlipidemic group. The addition of peanut to the diet did not change blood lipids, protrombin time, activated partial thromboplastin time or fibrinogen levels significantly both in the control and hyperlipidemic groups. It affected TF activities differently in both groups. It decreased brain and aorta TF activity but increased spleen and kidney TF activity in the control group. It led to significant increases in the TF activity of kidney, spleen and aorta and a significant decrease in the TF activity of brain in the hyperlipidemic group. *Conclusion* Peanut consumption improved GSH and HDL-C levels and decreased TBARS, without increasing other blood lipids in experimental hyperlipidemia. Nevertheless the mechanism of the effect of peanut consumption on the TF activity of tissues remains to be determined.

Key words hyperlipidemia – lipids – peanut – rat – tissue factor

Introduction

Peanuts are a rich source of B-vitamins, vitamin E, magnesium, copper and phosphorus [19]. In addition they are a source of plant protein, arginine, dietary fiber, and unsaturated fatty acids. Numerous bioactive substances (i.e. flavonoids, resveratrol and plant sterols) also are present in peanuts [19, 21]. Epide-

miologic studies published in the last decade have demonstrated a favourable effect of frequent consumption of nuts, including peanuts, on reducing the risk of coronary heart disease (CHD) [1, 14, 15, 22, 31].

Hyperlipidemia with increased concentrations of cholesterol and triacylglycerol-carrying lipoproteins is considered to be a cause of arteriosclerosis, with its dual sequel of thrombosis and infarction [4]. Traditionally nuts have been perceived by the general public as undesirable because of their high fat content. However the high fat content of nuts is largely unsaturated fats, which have favourable effects on blood lipids [20]. On the other hand, health professionals are still concerned whether hypercholesterolemic patients advised to eat nuts will have increased serum lipid levels.

Tissue factor (TF, Thromboplastin, Factor III) is considered to be the major regulator of normal haemostasis and thrombosis and plays a critical role in haemostasis in all tissues [5, 36, 40, 41]. Various tissues and body fluids have been known to have TF activity [3, 5, 40, 41] and increased levels of circulating TF activity is associated with hyperlipidemia [36].

Effects of peanut consumption on oxidant-antioxidant parameters and TF activities have not been studied in hyperlipidemia. The Nurses' Health Study of 86,016 female registered nurses has shown that frequent consumption of nuts, including peanuts (5 oz = 155 g of nuts/week), may reduce the risk of coronary heart disease [22]. In the present study, our aim was to adapt this dosage to rats to examine the effect of peanut consumption on blood lipids, glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation and heamotologic parameters. Moreover, we tested the hypothesis that, peanuts may confer additional protective effects that extend beyond their lipid-lowering effects, and one of these protective effects may be on the TF activities. Accordingly effects of peanut consumption on the TF activities of control and hyperlipidemic rat tissues were evaluated for the first time in the literature.

Table 1 Composition of diets fed to rats for 12 weeks

Dietary component [(g/g)%]	Groups			
	Control	Control + Peanut	Hyperlipidemic	Hyperlipidemic + Peanut
Cholic acid	–	–	0.4	0.4
Cholestreol	–	–	1.63	1.63
Sunflower oil	–	–	16.3	16.3
Peanut	–	0.63	–	0.63
Standart chow*	100	99.37	81.67	81.04

*Standard commercial chow content: 20% crude protein, 2.85% crude oil, 5.96% cellulose, 8% crude ash, 0.97% calcium, 0.5% phosphorous, 1.03% lycine, 0.33% methionine, 0.65% methionine + cysteine, 0.14% sodium, 1.13% linoleic acid, vitamin A, 9 000 IU/kg; vitamin D3, 2000 IU; vitamin E, 60 IU Ingredients used include: 45% wheat, 20% soybean bagasse, 6% corn, 3% sunflower bagasse, 10% bran flour

Methods

Animals and diets

All animal protocols were approved by the committee on the use of live animals in teaching and research, The University of Marmara. A total of 32 Wistar rats aged 8 weeks at the beginning of the experiment (initial body weight, 200–250 g), were used. The animals were obtained from the Marmara University Animal Laboratory. Animals were housed in cages in an environment-controlled room (room temperature, 22 ± 2°C; relative humidity, light/dark cycle, 12 h/12 h) with free access to food and water.

Rats were divided into 4 groups of 8 rats each: 1-Control group 2-Control+peanut group 3-Hyperlipidemic group 4-Hyperlipidemic+peanut group. Each group was fed on one of the following diets for 12 weeks: Control diet (Control group) or the control diet supplemented with 0.63% g peanut (Control+peanut group), or the control diet supplemented with 1.63% g cholesterol, 0.41% g cholic acid, 16.3% g sunflower oil (Hyperlipidemic group), or the hyperlipidemic diet supplemented with 0.63% g peanut (Hyperlipidemic+peanut group). The composition of the diets is shown in Table 1.

The diets were prepared once a week in the laboratory (except the control diet which was purchased from Denizeri Feed Manufacturer, Kocaeli, Turkey) and stored at a temperature of +4°C. Food consumption of the four groups was determined everyday throughout the experiment period.

Blood collection and tissue sampling

At the end of 12 weeks the rats were killed under urethane anaesthesia (1.25 g/kg) after overnight fasting. To minimize diurnal variations the rats were routinely killed between 07.00 and 08.00 h. Blood samples were removed from the heart and collected into tubes. Plasma and serum samples were separated by centrifugation. And stored at −20°C until lipid profile analysis except for (GSH), protrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen analyses, which were determined on the same day without delay. The tissues (lung, liver, brain, kidney, spleen, pancreas, heart and aorta) were excised, rinsed from blood in isotonic saline, blotted dry and weighed and stored at −20°C until the analysis of TF activity.

Biochemical assays

Commercial assay kits (Human) with code numbers; 10 028, 10 720P, 10 094, 10 084 were used for the determination of fasting serum total cholesterol (TC) [35], triacylglycerol (TG) [38], LDL-C [29] and HDL-C [18] levels respectively. Total lipid (TL), were determined by sulpho-phospho-vanilin [16] method.

Assay of GSH

Blood GSH concentration was determined according to the method of Beutler [7] using metaphosphoric acid for protein precipitation and 5′5′-dithiobis-2-nitro-benzoic acid for colour development.

Assay of TBARS

Lipid peroxidation was assayed by measuring malondialdehyde levels in serum. Malondialdehyde levels in serum were determined as TBARS according to the method of Yagi [39].

Haemostatic parameters

Commercial assay kits (Diagnostica Stago) with code numbers 00375, 00598 and 00608 were used for the determination of PT [6], APTT [27] and fibrinogen [17] levels, respectively.

Determination of tissue factor activity

The tissues were homogenized in 0.9% NaCI to obtain 10% g tissue homogenates except for aorta and brain which were prepared as 50% g homogenates. TF activities of tissue samples were evaluated according to Quick's one stage method using pooled plasma obtained from healthy subjects [23]. This was performed by mixing 0.1 ml tissue homogenate with 0.1 ml of 0.02 M $CaCl_2$, with the clotting reaction being started on addition of 0.1 ml of plasma. All reagents were brought to the reaction temperature (37°C) before admixture. Since the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity.

Statistical analysis

Data are presented as mean ± standard deviation. The differences were compared between study groups using two-way ANOVA with two factors (diet, peanut) for repeated measures followed by Bonferroni's multiple comparison test. Pearson correlation coefficients were computed to assess the relationships between the TF activities of the tissues and the parameters investigated. Differences with P values of 0.05 or less were considered significant. All statistical analysis were performed with the SPSS software for Windows, Version 11.5 [12].

Results

Prior to inducing hyperlipidemia (day 0), the groups were checked for the differences in weight and no significant difference in weights was found between any of the groups ($P_{ANOVA} > 0.05$). At the end of the experiment, the mean levels of body weight of the rats in control, control + peanut, hyperlipidemic and hyperlipidemic + peanut groups did not change significantly and they were found to be 312 ± 81; 286 ± 68; 295 ± 65; 304 ± 90 g, respectively. Daily food intake average of the rats in control, control + peanut, hyperlipidemic and hyperlipidemic + peanut groups were found to be 17, 16, 16 and 16 g respectively. Since food intake of each group could be determined instead of each rat, this data lacks statistical analysis.

In the control group peanut consumption did not cause any significant increase in blood lipids. It caused significant increase in GSH levels ($P < 0.01$) (Table 3). It did not alter PT, APTT and fibrinogen levels significantly in control group. It caused significant decrease in the TF activities of brain and aorta and significant increase in the TF activities of spleen and kidney (Table 4).

In the hyperlipidemic group, serum lipid concentrations and the TF activities of liver, kidney and spleen were found to be increased significantly whereas the TF activities of brain and aorta decreased significantly compared to the control group ($P < 0.01$). Furthermore GSH levels decreased whereas TBARS increased and tendency to coagulation was increased (Tables 2–4).

In the hyperlipidemic group peanut consumption significantly increased GSH, HDL-C levels and TF activities of kidney, spleen and aorta and significantly decreased TBARS and TF activity of brain ($P < 0.01$) (Tables 2–4). Significant correlations between TF activities and the parameters investigated are shown in (Table 5).

Discussion

In the Nurses' Health Study of 86,016 female registered nurses those consuming at least 5 oz (155 g) of peanut/week had a 35% lowering in nonfatal myo-

Table 2 Serum lipids of the four groups

	Groups			
	Control	Control + Peanut	Hyperlipidemic	Hyperlipidemic + Peanut
TC (mg/dl)	66.00 ± 11.38[a]	62.13 ± 3.56[a]	294.38 ± 27.41[b]	282.75 ± 28.23[b]
LDL-C (mg/dl)	15.00 ± 2.73[a]	16.00 ± 3.34[a]	66.88 ± 8.51[b]	63.00 ± 3.46[b]
HDL-C (mg/dl)	27.91 ± 3.06[a]	31.28 ± 5,08[a]	19.44 ± 0,61[b]	32.79 ± 2.03[a]
TG (mg/dl)	62.57 ± 5.10[a]	58.82 ± 6.52[a]	72.60 ± 3.90[b]	69.39 ± 1.85[b]
TL (mg/dl)	227.25 ± 48.27[a]	207.38 ± 29.02[a]	894.63 ± 68.97[b]	867.88 ± 62.04[b]

Values (n = 8) are means with standard deviations and were analysed by a two-way ANOVA, followed by the Bonferroni's multiple comparison test. Means not sharing a superscript letter in a row are significantly different $P < 0.01$. TC: Total cholesterol, TG: Triacylglycerols, LDL-C: Low density lipoprotein-cholesterol, HDL-C: High density lipoprotein-cholesterol, TL: Total lipid

Table 3 GSH and TBARS levels of the four groups

	Groups			
	Control	Control + Peanut	Hyperlipidemic	Hyperlipidemic + Peanut
GSH (% mg)	25.84 ± 3.42[a]	38.01 ± 3.71[b]	22.09 ± 2.28[a]	33.88 ± 3.64[b]
TBARS (nmol MDA/ml)	3.11 ± 0.41[a]	2.83 ± 0.27[a]	5.06 ± 0.41[b]	4.22 ± 0.27[c]

Values (n = 8) are means with standard deviations and were analysed by a two-way ANOVA, followed by the Bonferroni's multiple comparison test. Means not sharing a superscript letter in a row are significantly different $P < 0.01$. GSH: Glutathione, TBARS: Thiobarbituric acid reactive substances

cardial infarction compared with those eating less than 1oz of nuts [22]. In the present study, our aim was to adapt this dosage to rats to examine the effect of peanut consumption on blood lipids, an antioxidant and oxidant parameter, heamotologic parameters and the TF activities. For this purpose, we have calculated the equivalent dose of peanut for rats as 0.44 g peanuts/week, consuming human weight as 70 kg and rat weight as 200 g. Accordingly diets that contain 0.63% g peanut were prepared once a week so that rats would receive 0.44 g peanut/week.

It is generally accepted that a high-fat diet promotes obesity under ad libitum feeding conditions [9]. However in the present study, no significant changes in body weight of animals were observed, which may be attributed to the anorectic effect of cholic acid in diet [8].

At the end of 12 weeks, peanut supplementation did not increase blood lipids both in the control and hyperlipidemic groups. Rats fed hyperlipidemic diets supplemented with peanut had significantly higher HDL-C levels when compared with the hyperlipidemic group. Peanuts contain an array of healthful nutrients, but their lipid lowering property is primarily attributed to their fatty acid composition [25, 28]. Oleic acid which is the predominant dietary MUFA in peanuts is an effective LDL-C lowering agent but does not decrease HDL-C levels [25]. Peanuts are rich

Table 4 Haematologic parameters and the TF activities of tissues of the four groups

	Groups			
	Control	Control + Peanut	Hyperlipidemic	Hyperlipidemic + Peanut
PT (s)	16.50 ± 1.05	17.17 ± 1.47	16.33 ± 1.03	16.00 ± 0.63
APTT (s)	19.71 ± 1.70	19.33 ± 0.82	17.88 ± 1.13	18.13 ± 1.13
Fibrinogen (g/dl)	1.82 ± 0.28	1.83 ± 0.28	2.06 ± 0.24	1.98 ± 0.23
TF activity (s)				
Lung	22.25 ± 3.41	22.63 ± 3.38	20.25 ± 3.41	20.75 ± 2.55
Liver	110.13 ± 11.32[a]	110.88 ± 11.43[a]	82.13 ± 9.61[b]	82.13 ± 5.59[b]
Brain	48.88 ± 1.89[a]	54.88 ± 1.55[b]	52.63 ± 2.07[b]	57.25 ± 2.66[c]
Kidney	67.50 ± 1.60[a]	62.88 ± 2.03[b]	59.50 ± 1.41[c]	55.38 ± 1.60[d]
Spleen	67.63 ± 1.60[a]	64.38 ± 1.85[b]	51.88 ± 2.10[c]	48.38 ± 2.50[d]
Pancreas	33.13 ± 4.02	32.63 ± 6.57	30.38 ± 2.07	29.38 ± 3.20
Heart	30.88 ± 6.56	30.63 ± 5.80	28.50 ± 3.12	28.13 ± 2.53
Aorta	29.13 ± 1.36[a]	32.25 ± 1.83[b]	36.88 ± 1.46[c]	32.00 ± 2.00[b]

Values (n = 8) are means with standard deviations and were analysed by a two-way ANOVA, followed by the Bonferroni's multiple comparison test. Means not sharing a superscript letter in a row are significantly different $P < 0.01$. PT: Protrombin time, APTT: Activated partial thromboplastin time, TF Activity: tissue factor activity

Table 5 Significant correlations between tissue factor activities and investigated parameters

Control group	Correlation coefficient (r)
Brain TFa–LDL-C	−0.751*
KidneyTFa–HDL-C	−0.827*
KidneyTFa–LPO	0.781*
KidneyTFa–APTT	0.846*
Control + Peanut group	
Brain TFa–GSH	−0.750*
Pancreas TFa–Fibrinogen	−0.710*
Pancreas TFa–PT	0.882*
Hyperlipidemic group	
Liver TFa–APTT	−0.846**
Kidney TFa–PT	0.884*
Hyperlipidemic + Peanut group	
Lung TFa–LDL-C	0.712*
Brain TFa–TC	0.751*
Brain TFa–PT	0.831*
Liver TFa–Fibrinogen	−0.892**
Kidney TFa–Fibrinogen	−0.804*

TFa: Tissue factor activity, TC: Total cholesterol, LDL-C: LDL-cholesterol
HDL-C: HDL-cholesterol, GSH: Glutathione, LPO: Lipid peroxidation
PT: Protrombin time, APTT: Activated partial thromboplastin time
* $P < 0.05$ ** $P < 0.01$

sources of magnesium, folate, fiber, alpha tocopherol, copper and arginine all of which hold CVD risk-reducing properties [2, 19]. Low serum Mg concentrations can increase risk of CVD due to diminished lipoprotein lipase and lecithin cholesterol acyltransferase activity which results in hyperlipidemia [33]. The low lysine-arginine ratio of peanuts may promote serum cholesterol reduction and other physiological changes, which protect against atherosclerosis [10].

Nuts are rich sources of antioxidant substances (e.g. antioxidant vitamins, flavonoids, and phytosterols) [25]. More than 15 polyphenolics have been identified in peanuts and along with 80–140 mg/kg total tocopherols, these compounds may contribute to purported health benefits of peanuts [37]. Peanuts are rich sources of *p*-coumaric acid, which is an effective radical inhibitor in vitro [34, 37]. There is limited evidence suggesting that nuts improve plasma antioxidant potential [13, 25]. In fact to our knowledge, in the study presented here, the possible contribution of dietary peanut supplementation to serum antioxidant-oxidant status was investigated for the first time. Supplementation with peanut led to significantly higher GSH levels both in control and hyperlipidemic groups. When we compared the TBARS levels, there was a significant decrease in TBARS levels of the peanut given hyperlipidemic group compared with the hyperlipidemic group, which indicated that peroxidation reactions due to hyperlipidemia were to a great extent prevented by antioxidant constituents of the peanuts. Also the peanut consumption in the control group led to insignificantly lower levels of TBARS compared with the control group.

Many studies of atherosclerosis have indicated hyperlipidemia as a predisposing factor to vascular disease. The relationship between high lipid diets, hypercoagulability and the production of atherosclerosis and thrombosis is still a subject of conflicts. Using rats fed a high fat diet, Davidson et al. [11] presented evidence for hypercoagulability. In this study the PT and APTT of the hyperlipidemic rats showed a slight insignificant shortening compared to the control group. Olsen et al. [30] reported an increase in TG concentration which led to an increase in FVII activation after a high fat meal. The trend for the decrease in the PT and APTT of hyperlipidemic rats in this study might be due to the increased TG concentrations in this group. On the other hand when we compared the fibrinogen levels, hyperlipidemic rats were found to have insignificantly higher fibrinogen levels. Alterations in the fibrinolytic process in patients and animals have been shown by several workers to be associated with hyperlipidemia [24]. The elevated plasma fibrinogen levels are associated with an increased risk of cardiovascular disorders and may promote a prothrombotic or hypercoaguble state [32]. Dietary supplementation with peanut did not alter PT, APTT and fibrinogen levels significantly in control and hyperlipidemic groups.

Tissue factor (Thromboplastin) is considered to be the major regulator of normal haemostasis and thrombosis [36]. Exposure of vascular TF is the initial essential event in the extrinsic coagulation cascade [26]. TF plays a critical role in haemostasis in all tissues [3, 5, 40, 41] and exhibits a nonuniform tissue distribution with high levels in the brain, lung, and placenta, intermediate levels in the heart, kidney, intestine, uterus, and testes, and low levels in the spleen, thymus, skeletal muscle and liver [26]. In the present study, TF activity of tissues were determined instead of their levels. Accordingly, lung had the highest TF activity. The TF activity of aorta, heart, pancreas, brain, kidney and spleen followed it in decreasing ratios and liver had the lowest TF activity. Consequently, high-levels of TF corresponds to increased TF activity in a particular tissue.

An increased level of circulating TF activity is associated with diabetes mellitus, hyperlipidemia and smoking [36]. In this study the TF activities of various tissues were investigated using the Quick's one stage method. The TF activities of liver, kidney and spleen were found to be increased whereas the TF activities of brain and aorta decreased significantly in the hyperlipidemic group compared with the control group. Consistent with increased TF activities in our study, Sambola et al. [36] reported that patients with high LDL levels also had high levels of blood TF, suggesting a possible link with the predisposition towards thrombosis seen in patients with hyperlip-

idemia. The decrease in the TF activities of brain and aorta of hyperlipidemic rats may be due to a different haemostatic mechanism to protect these vital organs from the hyperlipidemic status.

Peanut supplementation affected the TF activities differently in control and hyperlipidemic groups. Peanut consumption in the control group decreased the TF activities of brain and aorta but increased the TF activities of spleen and kidney. A significant negative correlation was observed between the TF activity of brain and blood GSH levels in the peanut given control group (Table 3). The increased levels of GSH in this group might have prevented brain lipids from oxidation and therefore decrease the TF activity of brain. The oxidation of the lipids in the structure of TF may affect its activity. In the hyperlipidemic group peanut consumption led to increases in the TF activities of kidney, spleen and aorta and decrease in the TF of brain. The decrease in the TF activity of brain might protect this vital organ from the risk of thrombosis whereas the increased TF activities might provide additional haemostatic protections to these organs in order to prevent them from excessive bleeding.

The TF pathway is the primary physiological mechanism of initiation of blood coagulation [26]. Increased circulating TF activity has been shown to be associated with hyperlipidemia [36]. Our study has contributed to the literature by demonstrating that specific nutrients such as peanuts may also affect TF activity both in healthy and hyperlipidemic rats. However, whether this effect depends on the changes in the lipid profile is not clear and needs to be investigated.

Conclusion

The findings of this study suggest that peanut consumption may improve blood GSH, HDL-C levels, and decrease TBARS without increasing blood lipids in experimental hyperlipidemia. Nevertheless the mechanism of the effect of peanut consumption on the TF activities of tissues remains to be determined.

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