Antioxidant Activity Comparison of Walnuts and Fatty Fish

Chatrapa Hudthagosol DrPH*,
Ella Haddad DrPH**, Rewadee Jongsuwat PhD**

** Department of Nutrition, Faculty of Public Health, Mahidol University, Rajvithi Road, Ratchathewi, Bangkok, Thailand*
*** Department of Nutrition, Loma Linda University, Nichol Hall 1102, Loma Linda, CA, USA*

Background: *Walnuts and fatty fish contain high amounts of polyunsaturated fatty acids, which have been shown to decrease the incidence of cardiovascular disease. Walnuts and fatty fish also contain other nutrients, such as antioxidants, that contribute to the reduction of cardiovascular disease.*
Objective: *The purpose of the present study was to compare the effects of dietary walnuts and fatty fish on the plasma and urine oxygen radical absorbance capacity (ORAC) values.*
Material and Method: *Twenty-five subjects participated in this randomized 3 x 3 crossover study, which was performed under controlled metabolic feeding conditions. Subjects consumed 3 isoenergetic diets and each diet was consumed for 4 weeks: a control diet (no nuts or fish), a walnut diet (1.5 oz/day of walnuts, 6 times/week) and a fish diet (8 oz/week of salmon). Blood specimens were collected at baseline and at the end of each diet period.*
Results: *The results showed that the plasma hydrophilic ORAC was significantly higher in the walnut diet compared with the control diet and the fish diet ($p < 0.0001$). In addition, the urine ORAC was significantly higher in the walnut diet and the fish diet compared with the control diet ($p < 0.0001$). Moreover, the hydrophilic/lipophilic ORAC for the food itself was significantly higher in the walnut diet compared with the control diet and the fish diet ($p < 0.0001$).*
Conclusion: *The present results suggest that walnuts have a large antioxidant capacity; therefore, including walnuts in the daily diet may be beneficial to maintain an antioxidant status in the body.*

Keywords: *Walnuts, Fatty fish, Polyunsaturated fatty acids, Oxygen radical absorbance capacity, Cardiovascular disease*

J Med Assoc Thai 2012; 95 (Suppl. 6): S179-S188
Full text. e-Journal: *http://jmat.mat.or.th*

The human body is constantly being exposed to reactive oxygen species, which are characterized by the presence of molecules that carry unpaired electrons that may damage cellular molecules and structures. These reactive oxygen species can trigger other reactions in the cells and result in significant damage to the entire tissue, which is known as oxidative stress[1-3]. Thus, it is important that the body gain dietary nutrients to combat the effects of reactive oxygen species. At the front line of defense are antioxidant enzymes, which directly interact and stabilize highly reactive oxygen molecules. Other antioxidants can be derived from the daily diet, including fruits, vegetables, nuts and fish[4-6].

Due to the great variation and selection of food items in our diet, there is a need to determine the actual antioxidant capacity of each particular food item[7]. To date, several databases have been constructed to present different food items with their corresponding antioxidant capacities, which have been measured by three common approaches: the reduction of ferric molecules, the Trolox equivalent and the amount of oxygen radicals[3]. The actual concentration of the antioxidant does not directly correlate with its antioxidant capacity, which is mainly due to the reduction and oxidation reactions that occur between other molecules that are derived from other food items in a meal[8,9]. There are also variations in the chemical compositions of the same species of fruits, depending upon the geographic origin of the species.

There is a growing interest in two particular food items that are considered to impart antioxidant effects after consumption: walnuts and fish. Walnuts and fish are two food sources that have been determined to contain polyunsaturated fatty acids that could actually be beneficial to the body by

Correspondence to:
Hudthagosol C, Department of Nutrition, Faculty of Public Health, Mahidol University, Rajvithi Road, Ratchathewi, Bangkok 10400, Thailand.
Phone: 0-2354-8539
E-mail: phchatrapa@mahidol.ac.th

imparting antioxidant effects after consumption[6,8,10,11]. Studies have previously shown that the ingestion of nuts correlates with a lower incidence of heart disorders[12-16]. This particular effect is based on the reduction in the level of low-density lipoproteins, which are replaced by fatty acids present in nuts[16,17]. This significant reduction results in the prevention of cardiovascular disorders, which are primarily caused by an accumulation of lipids in the blood. Nuts are also known to contain other beneficial components, such as fiber and essential vitamins[18]. The active components in nuts responsible for lowering plasma lipid levels are polyphenols[8,19]. The specific physiological effects of walnut consumption are rapid, whereas plasma polyphenol levels increase approximately 90 minutes after consumption of the nuts[10]. The antioxidant capacity of walnuts was determined to be at its highest level within 150 minutes after ingestion. The amount of plasma lipids also markedly decreased within 90 minutes after consumption of a meal containing walnuts[10].

Fatty fish or fish oils are known to contain omega-3 fatty acids, which contain a carboxyl group that are beneficial to the human body[6,11]. Numerous research reports in the last few decades have described the positive effects of omega-3 fatty acids on the cardiovascular system[20,21]. One of the most studied fatty acids in fish is alpha-linolenic acid, which is also present in walnuts[22]. Alpha-linolenic acid was reported to decrease triglyceride concentrations in plasma by approximately 50%[23]. In addition, the decrease in triglycerides was reported to be more dramatic when the subject showed a high baseline triglyceride level[24]. Studies have estimated that administration of approximately one gram of fish oil each day could decrease triglyceride levels by eight milligrams per deciliter. Furthermore, individuals with high baseline triglyceride levels may experience a decrease in triglyceride levels by almost 27 milligrams per deciliter[24].

Experts have recommended that fish be included in at least two meals per week, but consumers should be aware that not all types of fish contain the same amount of linolenic acid. For example, cod and catfish only contain a minimal amount of linolenic acid[20,22], whereas salmon contains five times as much linolenic acid as catfish. Thus, it is helpful to know which fish species may provide more of the beneficial fatty acids for cardioprotective and antioxidative functions[21,23].

Considering the research already performed on fish and walnuts, we were interested in determining which of the two food items would provide more favorable effects upon people's health. The present study described the comparative effects of walnuts and fatty fish. The present study followed a recent report by Rajaram et al[6]. The effects of walnuts and fatty fish on serum lipid levels were examined in individuals with either normal or high cholesterol levels. The randomized study was conducted in 25 adults who were given one of three types of isoenergetic diets for four weeks. The isoenergetic diet was based on a total fat content of 30% and a total saturated fat content of less than 10%. The control isoenergetic diet did not include any nuts or fish in the meals. The walnut diet contained a precise amount of walnuts, which was equivalent to 10.1 mJ or 42.5 grams. The third isoenergetic diet was the fish diet, which was primarily comprised of 113 grams of salmon delivered twice per week. The fasting serum lipid levels of the participants were collected before and directly after the experimental period. The clinical study showed that the subjects who were given the walnut diet presented significantly lower cholesterol and low-density lipoprotein levels compared with subjects who were given the control or the fish diet. The lipid levels of the subjects given the fish diet, however, were lower than the lipid levels in the control subjects[6].

Similar results were obtained from another study by Tapsell et al[17] which included 30 grams of walnuts in the daily diet of patients diagnosed with type 2 diabetes. The cholesterol levels of these subjects decreased by approximately 10% after consuming walnuts for at least three months. Taken together, the Rajaram et al and Tapsell et al studies suggest that walnuts have a greater antioxidant capacity than fish and other varieties of nuts. In addition, a chemical analysis showed that specific fractions of walnuts provide variations in antioxidant capacity. According to Oliveira et al[9] the defatted portion of the walnut carried a greater antioxidant capacity than the extracted oil. Interestingly, the antioxidant effects of walnuts were actually determined to be stronger than alpha-tocopherol, which resulted in the oxidation of low-density lipoproteins through the mediation of copper ions in plasma[4,19].

Oxidative damage is considered to be a major causative factor in the development of cancer and certain cardiovascular disorders[4,7,25]. Oxidative damage may be due to both environmental and lifestyle behavior, such as exposure to air pollutants and smoking. Oxidative damages may accumulate

in the body and a decrease in the capacity of the body to repair and replace damaged cells can result in a variety of medical disorders[25]. The presence of free radicals, especially oxygen species, may damage both cellular and molecular structures that are essential for the normal physiological functioning of tissues, organs and the rest of the human body. The antioxidant capacity of walnuts and fatty fish has been investigated by several medical researchers and a positive correlation has been established between the amount of walnuts and fatty fish consumed and the plasma levels of lipoproteins. Comparative analysis, however, has shown that walnuts have a greater effect in lowering the level of low-density lipoproteins compared with fatty fish[6]. To date, no studies have compared the influence of walnuts and fatty fish consumption on the antioxidant capacity of plasma and urine. The purpose of the present study was to compare the effects of dietary walnuts and fatty fish on plasma and urine oxygen radical absorbance capacities (ORACs).

Material and Method

Subjects

Twenty-five volunteers participated in the present study. Recruitment was achieved through advertisements in Loma Linda, California and the surrounding communities. All participants were screened through a multi-stage approach: a telephone screening, an informational group meeting, two independent interviews by investigators and a preliminary fasting blood lipid test.

Subjects were between the ages of 23 to 65 years (12 females and 13 males) and they were non-dyslipidemic, non-smoking adults with negligible alcohol intake (2 drinks per week or less). In addition, subjects did not have any significant weight change during the previous six months. Subjects were excluded if they were taking vitamin E supplements, fish or flaxseed oil, had a high daily intake of nuts (> 2 times/week), drank caffeinated beverages (> 3 times/day), had endocrine or metabolic disturbances or any chronic diseases and/or had a serum triglyceride level above 300 mg/dL and serum cholesterol less than or equal to 300 mg/dL.

All participants were required to maintain a consistent level of physical activity throughout the study. All eligible subjects signed an informed consent before filling out medical and dietary questionnaires and having a screening serum cholesterol test. The present study protocol was approved by the Institutional Review Board (IRB; Loma Linda University, Loma Linda, CA). Subjects received a modest monetary incentive for completing the study. A total of twenty-five subjects completed the study (Tables 1 and 2).

Study design

The effects of walnuts compared with fatty fish were assessed by plasma, urine and food ORACs and compared using a controlled, single-blind, randomized crossover (3 x 3 Latin-square) design. Twenty-five subjects were given one-week run-in periods to obtain a stable baseline. During the first week run-in period, all subjects were given the typical US diet following the guidelines of the Dietary Guidelines for Americans, with restrictions on intake of n-6 fatty acids. Following the run-in period, subjects were assigned to follow one of the three diets: control, walnut or fish for 4 weeks. All subjects were randomly stratified on the basis of age, gender, body mass index (BMI) and baseline serum cholesterol. There was a weekend break between the three diet periods (Table 3).

Dietary intervention

Throughout the study, participants received all their meals from the research staff. Breakfast and dinner were served daily at the UD. Register Nutrition Research Kitchen located at Loma Linda University and were eaten in the presence of one of the senior researchers. Lunches and snacks were packed and distributed at breakfast. All foods and drinks were weighed and apportioned for each subject. Meals consisted of food items prepared in customary ways and followed a 9-day weekday and 2-day weekend menu cycle. To accommodate the different caloric needs of study participants, the portions of each meal were based

Table 1. Characteristics of the study subjects at entry

Characteristics	Mean (range)
n	25
Age (years)	33 (23-65)
Body mass index (kg/m^2)	24.8 (18.7-36.6)
Body weight (kg)	70.9 (51.5-115.8)
Serum lipids, mmol/L	
Total cholesterol	5.41 (3.4-7.76)
Low-Density Lipoprotein	3.53 (1.82-5.66)
High-Density Lipoprotein	1.43 (0.88-2.33)
Triglycerides	1.25 (0.66-3.33)

To convert cholesterol and triglyceridefrom mmol/L to mg/dL, multiply by 38.67 and 88.57, respectively

Table 2. Mean body weight and body mass index in each diet period[+,++]

	Walnut diet	Fish diet	Control diet
Body weight (kg)	71.80 ± 3.06	71.50 ± 3.05	70.10 ± 3.03
Body mass index (kg/m^2)	25.16 ± 0.77	25.06 ± 0.77	25.08 ± 0.77

[+] Walnut diet; Fish diet; Control diet, [++] All values are mean ± SD; n = 25; Differences were not statistically significant (mixed-linear models and Tukey's least-squares means test)

Table 3. Study design

Week 0	Experimental Design													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Run-in period;	1	Control Group			W/O		Walnut Group			W/O		Fish Group		
subjects will be	2	Walnut Group			W/O		Fish Group			W/O		Control Group		
randomly assigned	3	Fish Group			W/O		Control Group			W/O		Walnut Group		
to one of six study	4	Control Group			W/O		Fish Group			W/O		Walnut Group		
sequences	5	Walnut Group			W/O		Fish Group			W/O		Control Group		
	6	Fish Group			W/O		Walnut Group			W/O		Control Group		
Blood Draw						XX				XX		XX		

W/O = Wash out

on individual caloric needs for weight maintenance.

The experimental diets (control, walnut and fish) were isoenergetic and provided an equivalent amount of total fat (32-33% of the total calories) and saturated fat (8-9% of the total calories). At the 2,400 calorie level, the walnut diet included 1.5 oz of walnuts (6 times/week), the fish diet included 8 oz of salmon per week (4 oz twice a week) and the control diet included foods other than nuts and fatty fish. The nutrition composition of the treatment diets is shown in Table 4.

The experimental diets did not include n-3 rich foods other than walnuts in the walnut diet and fish in the fish diet. Excluded foods were soybeans, soy products, soy oil, canola oil, flaxseed, and flaxseed oil. Duplicate samples of the study diets were randomly selected on 10 days during the study period. The samples were blended, and a 5% portion of each diet was kept frozen at -80°C.

During all three diet periods, the subjects were provided diaries to record their lifestyle habits, which included activities, any signs of illness, medications used and any deviation from their experimental diets. Assessment of dietary compliance was performed by direct observation during the meal times and weekly examination of the subjects' diaries. Body weight without shoes was recorded daily during the run-in period and twice a week thereafter. Daily energy intake was recorded and adjusted when necessary to maintain body weight.

Biological data collection and laboratory analyses

Blood was drawn from each subject a total of 8 times on two alternate days. All blood draws were performed at the Department of Nutrition Assessment Laboratory located at Loma Linda University, and subjects arrived on the assigned days after fasting for a minimum of 12 hours. An experienced phlebotomist drew the blood and the samples were centrifuged, aliquoted for the various assays and stored immediately at -80°C in the Nutrition Biochemical Laboratory located at Loma Linda University. All assays were performed at the end of the present study to control for between-assay variations. Extra aliquots were stored for additional outcomes of interest that might arise during or after the completion of the present study.

The oxygen radical absorbance capacity (ORAC) assay

The ORAC assay depends on the free radical damage to a fluorescent probe through the change in its fluorescence intensity. The change in fluorescence intensity is an indication of the degree of free radical damage. Antioxidants will inhibit free radical damage, which is reflected by a lack of change in the probe

Table 4. Nutrition composition of the treatment diets[+]

	Control	Fish	Walnut
Energy (kcal)	2,400	2,400	2,400
Total Fat (g)	79.02	78.00	82.81
Total Carbohydrates (g)	346.89	347.39	359.28
Total Protein (g)	87.36	88.88	86.76
Cholesterol (mg)	339.05	343.07	281.54
Total Saturated Fatty Acids (SFA) (g)	25.07	24.07	20.51
Total Monounsaturated Fatty Acids (MUFA) (g)	36.12	35.80	26.52
Total Polyunsaturated Fatty Acids (PUFA) (g)	11.48	11.93	29.30
Total Trans-Fatty Acids (TRANS) (g)	2.71	2.55	2.24
Omega-3 Fatty Acids (g)	1.09	2.07	4.76
Total Dietary Fiber (g)	26.33	26.15	29.36
Total Vitamin A Activity (Retinol Equivalents) (mcg)	1,427.14	1,442.11	1,429.52
Vitamin D (calciferol) (mcg)	2.82	6.24	2.60
Vitamin E (Total Alpha-Tocopherol) (mg)	7.26	7.70	7.82
Gamma-Tocopherol (mg)	7.43	6.92	16.12
Vitamin K (phylloquinone) (mcg)	141.20	118.54	136.09
Vitamin C (ascorbic acid) (mg)	211.77	206.99	227.61
Thiamin (vitamin B1) (mg)	1.92	2.00	2.07
Riboflavin (vitamin B2) (mg)	2.22	2.34	2.21
Niacin Equivalents (mg)	25.28	25.17	25.04
Vitamin B-6 (pyridoxine, pyridoxyl and pyridoxamine) (mg)	2.09	2.36	2.25
Dietary Folate Equivalents (mcg)	740.48	736.53	795.76
Vitamin B-12 (cobalamin) (mcg)	3.95	4.58	3.48
Zinc (mg)	11.23	10.41	11.61
Copper (mg)	1.71	1.81	2.37
Manganese (mg)	4.07	4.17	5.45
Potassium (mg)	3,539.76	3,700.97	3,703.80

[+]Calculated by The Nutrition Data System for Research (NDS-R) software, version.03_31. 2000

Table 5. Descriptive statistics of the plasma, urine and food ORACs[+]

		Plasma ORAC (μmol/L)		Diet ORAC (μmol Trolox equiv/g)		Urine ORAC (μmol/L)
	n	Hydrophilic ORAC	Lipophilic ORAC	Hydrophilic ORAC	Lipophilic ORAC	Hydrophilic ORAC
Control	23	170.71 ± 1.48	196.97 ± 1.47	1,164.15 ± 17.86	1,155.96 ± 36.47	81.07 ± 9.56
Fish	24	172.29 ± 1.65	198.19 ± 1.91	1,284.23 ± 21.53	1,220.43 ± 38.0	92.99 ± 12.39
Walnut	23	178.60 ± 1.49	200.95 ± 1.95	5,674.18 ± 95.33	5,622.69 ± 178.26	94.14 ± 14.45

[+] Data presented as mean ± SD

fluorescence. This assay utilizes a biologically relevant radical source and is the only method that combines both the inhibition time and degree of inhibition into a simple quantity.

Extraction of plasma samples

Plasma or serum samples that had been stored at -80°C were thawed slowly, mixed on a vortex and centrifuged if needed. Two hundred microliters of plasma or serum was transferred to a glass tube followed by the addition of 400 μL of ethanol and 200 μL of water and the solution was mixed. One milliliter of hexane was then added and mixed. The mixture was left to sit for 1-2 min or until two layers appeared before being centrifuged for 10 minutes at 3,000 rpm. Five hundred microliters of the hexane layer was removed

and added to a separate amber tube. An additional 500 μL of hexane was added to the original tube, mixed, allowed to settle for 2 min and then centrifuged for 10 minutes at 3,000 rpm. Another five-hundred microliters of the hexane layer was removed and combined with the first extract. The combined hexane extracts were dried down under nitrogen in preparation for the lipophilic $ORAC_{FL}$ analysis. Any remaining hexane following hexane extraction of the aqueous plasma sample was removed by drying under nitrogen. Then, 800 uL of 0.5 M perchloric acid was added to precipitate the protein. The sample was then centrifuged for 10 minutes at 3,000 rpm. The clear supernatant was removed and added to a new amber tube.

Extraction of the urine samples

Urine samples were taken out of the freezer, thawed, vortexed and centrifuged. Four hundred microliters of the urine samples was pipetted into glass tubes, 1 ml of hexane was added and the mixture was vortexed for five minutes. The mixture was then centrifuged for 10 minutes at 3,000 rpm (4°C). Five hundred microliters of hexane was removed from the hexane layer and transferred to a new tube. An additional 500 μL of hexane was added to the original tube, vortexed for 2 minutes and centrifuged at 3,000 rpm at 4°C. Eight hundred microliters of hexane from the hexane layer was removed and combined with the first extract. The combined hexane extracts were dried for the lipophilic ORAC analysis.

Eight hundred microliters of 0.5 M perchloric acid was added to the original tubes to precipitate the protein. The sample was then centrifuged for 10 minutes at 3,000 rpm at 4°C. The clear supernatant was removed and added to new tubes. The supernatant was stored if it was not instantly used.

Extraction of food samples

Five grams of food samples were distributed into 15-mL screw-cap tubes. Ten milliliters of hexane was then added to the tube, vortexed and centrifuged for 10 minutes. Nine milliliters of hexane from the hexane layer was removed and transferred to a glass screw-cap tube. The sample was mixed and 8 mL of hexane was added, vortexed and centrifuged for 10 minutes. Seven milliliters of hexane from the hexane layer was removed and combined with the first extract. The combined hexane extracts were dried and stored in a freezer for the lipophilic ORAC analysis.

A ten milliliter mixture of acetone, water and acetic acid (a ratio of 70: 29.5: 0.5, respectively) was added to the original tubes, vortexed and heated at 37°C for 5 minutes. The tubes were then held at room temperature for 10 minutes with occasional shaking. The tubes were centrifuged for 15 minutes and stored in a freezer if they were not instantly used.

Running the ORAC assay

For the hydrophilic assay, the dried hexane extract was dissolved in 5 mL of acetone. Twenty-five microliters was removed and diluted with 1 mL of a 7%. Randomly methylated β-cyclodextrin (RMCD) solution (50% acetone/50%water, v/v). For the lipophilic assay, a 7% RMCD solution was used as a blank and to dilute the Trolox standards. Along with the standards and samples, a 7% RMCD solution (forty microliters) was pipetted into the wells of a 48-well microplate. Four hundred microliters of a fluorescein solution was added to each well and mixed on the shaker. The microplate was then placed in the microplate reader, which was kept warm at 37°C and programmed to inject 150 μL of fresh 2, 20-Azobis (2-amidino-propane) dihydrochloride (AAPH) into each well.

In the hydrophilic ORAC assay, a 100 uL sample was diluted in 10 mL of phosphate buffer in glass tubes. Phosphate buffer was used as the blank and to dilute the Trolox standards. Forty microliters of phosphate buffer (0.15M, pH 7.8) along with the standards and samples was pipetted into the wells of a 48-well microplate. Four hundred microliters of a fluorescein solution was added to each well and mixed on the shaker. The microplate was then placed in the instrument, which was kept warm at 37°C and programmed to inject 150 uL of fresh AAPH into each well.

Statistical analysis

All data analyses were performed using SAS software, version 8.0 (SAS Institute Inc., Cary, NC) by the present study statistician and verified by the principal investigator. Results are expressed as mean $\pm$ standard deviation (SD) of the mean concentration in plasma, urine and foods. A mixed-model approach was used to compare plasma and the urine hydrophilic/lipophilic ORAC among the three diets (control, fish and walnut), which controlled for the period effect (phase 1, 2, 3) and baseline measurement. Subject nested within sequence was also added into the model as a random effect. The Kenward-Roger method was employed to estimate the denominator degrees of freedom for tests of fixed effects. The Tukey-Kramer Honestly Significant

Difference (HSD) test was performed to detect significant pair-wise differences among the three diets.

Results

The plasma hydrophilic/lipophilic ORAC

For the hydrophilic ORAC measurements, there was a highly significant diet effect ($p < 0.0001$). The hydrophilic ORAC was significantly higher in the walnut diet compared with the control diet ($p < 0.0001$) and the fish diet ($p < 0.0001$). Interestingly, there was no significant difference in the hydrophilic ORAC between the control diet and the fish diet ($p = 0.426$). For the lipophilic ORAC measurements, there was no significant difference between the three diets ($p = 0.090$) (Table 5).

The urine ORAC

There was a highly significant diet effect in the urine ORAC ($p < 0.0001$). Indeed, the urine ORAC was significantly lower in the control diet compared with the fish diet ($p < 0.0001$) and the walnut diet ($p < 0.0001$). There was no significant difference in the urine ORAC between the fish diet and the walnut diet ($p = 0.838$).

The foods hydrophilic/lipophilic ORAC

For the hydrophilic ORAC measurement, there was a highly significant diet effect ($p < 0.0001$). The hydrophilic ORAC was significantly higher in the walnuts compared with the control diet ($p < 0.0001$) and the fish ($p < 0.0001$). There was no significant difference in the hydrophilic ORAC, however, between the control diet and the fish ($p = 0.789$).

For the lipophilic ORAC, there was a highly significant diet effect ($p < 0.0001$). The lipophilic ORAC was significantly higher in the walnuts compared with the control diet ($p < 0.0001$) and the fish ($p < 0.0001$). There was no significant difference in the lipophilic ORAC, however, between the control diet and the fish ($p = 0.977$).

Discussion

Walnuts and fatty fish are known for their high amounts of polyunsaturated fatty acids, which are considered to be beneficial compounds for the human body. Studies have reported that consumption of nuts is strongly associated with a decrease in the incidence of cardiovascular disease[18]. This specific result is mainly influenced by a decrease in the concentration of low-density lipoproteins in the blood, which are substituted by the fatty acids found in nuts[26]. The significant decrease in low-density lipoproteins influences the development of cardiovascular diseases, which are generally derived through the accumulation of fat molecules in the blood. Nuts, such as walnuts, have also been determined to be composed of other essential components, including fiber, essential vitamins, phytosterols, and polyphenols. The main influential components of nuts that decrease plasma lipid levels are the polyphenolic compounds[8]. Specific physiological results of walnut ingestion are quick, whereas the amount of plasma polyphenols does not increase until one hour after ingestion of nuts[10]. Moreover, the concentration of antioxidant capacity was greatest at within 150 minutes after the consumption of nuts. The concentration of lipids in the plasma was also significantly decreased within 90 minutes following ingestion of a meal that contained walnuts. Consistent public health messages that have emerged for the prevention of heart disease is increasing n-3 fatty acids from plants such as walnuts in the diet of Americans[6,8].

Fatty fish contain omega-3 fatty acids, which are composed of chains of carbon atoms that are beneficial to the body[11]. Research investigations in this area have been increasing for a number of years, and studies have shown beneficial effects of fatty fish on the cardiovascular condition of human subjects. One of the most investigated fatty acids is linolenic acid, which can also be derived from walnuts[22]. Linolenic acid was found to lower the concentration of triglycerides in the blood by half its magnitude[23]. Moreover, the lowering of the triglyceride concentration was more effective if the individual had an elevated baseline concentration[24]. Experts have calculated that the administration of approximately one gram of fish oil daily can decrease triglyceride levels by approximately 8 milligrams per deciliter. In addition, individuals with elevated baseline levels of triglycerides may experience an even greater decrease in triglyceride concentrations by approximately 27 milligrams per deciliter.

The inclusion of fish into at least two meals per week has been recommended; however, not all species of fish contain equivalent amounts of linolenic acid[11]. For example, codfish and catfish only contain a small amount of linolenic acid, whereas salmon contains 500% more linolenic acid than most fish species. Thus, it is important to recognize which types of fish may deliver significant amounts of healthy fatty acids for cardiovascular protection and antioxidant benefits. Based on previous reports, walnuts and fatty fish can

elicit beneficial effects in the body. In a recent article by Rajaram et al[6] the consumption of walnuts and fatty fish was investigated to observe the effects on the serum lipid concentration in human subjects who showed either a normal or high level of cholesterol. A random case-controlled study was performed using 25 adults who were administered one of three types of isoenergetic diets for an entire month. The isoenergetic diet was composed of 30% fat and 10% saturated fatty acids. The control isoenergetic diet was not composed of any nuts, whereas the walnut diet was composed of a measured amount of walnuts[17]. A third isoenergetic diet was composed of approximately 113 grams of fish, which was administered twice a week. The fasting serum lipid levels of the participants were collected before and directly after the experimental period. The clinical study showed that the subjects who were given the walnut diet had significantly lower cholesterol levels and low-density lipoprotein levels than did the subjects who were given the control diet or the fish diet. In addition, the lipid levels were lower in the subjects given the fish diet compared with the control subjects. The antioxidant capacity of walnuts and fatty fish has been determined by several investigators and a positive correlation has been observed between the amount of walnuts and fatty fish eaten and the plasma levels of lipoproteins. In addition, a comparative analysis has reported that walnuts have a more significant effect in decreasing the level of low-density lipoproteins than fatty fish.

In the present study, the authors compared the effects of dietary walnuts and fatty fish on plasma and urine ORACs. The results showed that the plasma hydrophilic ORAC was significantly higher in the walnut diet compared with the control diet and the fish diet. In addition, the urine ORAC was significantly higher in the walnut diet and the fish diet compared with the control diet. Moreover, the hydrophilic/lipophilic ORAC was significantly higher in the walnut diet compared with the fish diet and the control diet.

In summary, the walnut diet appeared to have a higher antioxidant capacity than the fish diet. Walnuts are rich in many beneficial nutrients, including vitamin E (gamma-tocopherol) and a wide range of polyphenolic compounds, which may potentially contribute to the antioxidant capacity. The health effects of walnuts may not only rely on blood lipid changes but also on antioxidant nutrients and bioactive components. Although consumption of walnuts increased the plasma and urine ORAC levels, further studies are needed.

Potential conflicts of interest

None.

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การเปรียบเทียบสารต้านอนุมูลอิสระของวอลนัทและปลาที่มีไขมัน

ฉัตรภา หัตถโกศล, เอลลา ฮาดาด, เรวดี จงสุวัฒน์

ภูมิหลัง: *วอลนัทและปลาที่มีไขมันสูงประกอบไปด้วยกรดไขมันไม่อิ่มตัวเชิงซ้อนสูง ซึ่งสามารถแสดงว่าช่วยลดอุบัติการณ์ของการเกิดโรคหัวใจ วอลนัทและปลาที่มีไขมันสูงยังคงประกอบไปด้วยสารอาหารอย่างอื่นเช่น สารต่อต้านอนุมูลอิสระ ซึ่งก่อให้เกิดการลดลงของโรคหัวใจ*

วัตถุประสงค์: *จุดประสงค์ของการศึกษานี้คือ การเปรียบเทียบผลของการบริโภคอาหารประเภทวอลนัทกับปลาที่มีไขมันสูง โดยดูจากการวัดค่าศักยภาพของสารต้านอนุมูลอิสระที่จะทำให้อนุมูลกลายเป็นกลาง (oxygen radical absorbance capacity หรือ ORAC) ในพลาสมาและปัสสาวะ*

วัสดุและวิธีการ: *อาสาสมัครทั้งหมดยี่สิบห้าคนถูกสุ่มและทำการทดลองแบบไขว้กัน 3 x 3 และอยู่ภายใต้การควบคุมอาหารที่ได้รับอาสาสมัครแต่ละกลุ่มได้รับอาหาร 3 ประเภทโดยแต่ละประเภทใช้เวลา 4 สัปดาห์: อาหารควบคุม (ไม่มีวอลนัทและปลา) อาหารที่มีวอลนัท (1.5 ออนซ์/วันของวอลนัท, 6 ครั้ง/อาทิตย์) และอาหารที่มีปลาที่มีไขมันสูง (8 ออนซ์ต่อสัปดาห์โดยใช้ปลาแซลมอน) ตัวอย่างเลือดจะถูกเก็บก่อนการทดลองและสิ้นสุดการทดลองในแต่ละช่วง*

ผลการศึกษา: *ผลการทดลองพบว่าค่าของพลาสมาไฮโดรฟิลิกมีค่าสูงในกลุ่มของอาหารที่มีวอลนัทเมื่อเทียบกับกลุ่มอาหารควบคุม และกลุ่มอาหารที่มีปลาที่มีไขมันสูงอย่างมีนัยสำคัญทางสถิติ ($p < 0.0001$) นอกจากนี้ค่าของสารต้านอนุมูลอิสระที่จะทำให้อนุมูลกลายเป็นกลางในอาหารกลุ่มของอาหารที่มีวอลนัทสูงกว่ากลุ่มอาหารควบคุมและกลุ่มอาหารที่มีปลาที่มีไขมันสูง ($p < 0.0001$)*

สรุป: *จากผลการศึกษานี้ระบุว่าวอลนัทมีค่าของสารต่อต้านอนุมูลอิสระสูง ดังนั้นการที่บริโภควอลนัทในแต่ละวันอาจช่วยส่งผลให้มีการรักษาปริมาณสารต่อต้านอนุมูลอิสระในร่างกายปลอดภัยและแข็งแรง*